# ON TWO LITTLE-KNOWN MICROSPORIDIA OF MARINE FISHES

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(With 3 Figures in the Text)

Parasites of marine fishes have attracted, comparatively speaking, but little attention among scientists. This is true especially for the parasites of those fishes which live in the open seas, and are therefore less accessible to investigation than their relatives inhabiting coastal waters. On many occasions, when chance has brought such a parasite to the attention of a research worker, the scarcity of data allowed no more than mere recording of the existence and a brief description of the parasitic species. Now, when investigations into the parasite fauna of marine fishes are being intensified, it is possible to add to our knowledge of such forgotten species of parasites, in order to appreciate more clearly their possible importance to their hosts and, indirectly, to the economics of the stocks of our food fishes.

This paper deals with two such species, both belonging to the microsporidian family Nosematidae. They are: *Nosema branchiale* Nemeczek, 1911, and *Plistophora hippoglossoideos* Bosanquet, 1910.

## (1) **Nosema branchiale** Nemeczek, 1911

This parasite was discovered by Nemeczek on the gills of haddock (*Gadus aeglefinus* L.) in the fish-market of Vienna. The discoverer, who at the time studied the possibility of man being infected with fish Protozoa, dealt mainly with the locally caught freshwater fishes. In his description of the species Nemeczek (1911) states, however, that he also examined some fish which were imported frozen from Russia and America. It is probable, therefore, that the haddock infected with this microsporidian came either from the northern coast of Russia or the Atlantic coast of North America. The parasite was observed on two occasions separated by two years, in the course of almost daily investigations, and the discoverer, not unnaturally, concluded that it was extremely rare.

The site of infection was the gill filaments, on which the parasite formed 'globular, very small structures, 0·2–0·5 mm. in diameter and whitish-grey in colour'. On one occasion cysts 'up to 1 mm. large, ovoid and with slightly blunted ends' were recorded. The only stage described by the author were the spores, oviform in shape, containing strongly granular sporoplasm, readily observable vacuole and the polar capsule with a filament, which could be extruded by pressing the coverslip. The dimensions of the spores are shown in Table 1.

*N. branchiale* was not recorded again until some twenty years after its discovery. During these years, and even later, workers concerned with Microsporidia continued to refer to it as occurring in the fish-market of Vienna (Kudo, 1924; Reichenow,

1932; Schäperclaus, 1954). Very few records of *N. branchiale* seem to have appeared in the literature between the date of its description and the present day. Bazikalova (1932) reported it from cod (*Gadus callarias* L.) and haddock of the Barents Sea, stating that it occurs more commonly in the latter than in the former. It was impossible to consult Bazikalova's work, but it is mentioned by Dogiel (1936), who found three cod out of five examined in the Barents Sea to be infected with *N. branchiale*. In one of the infected fishes the cysts formed by the parasite were

Table 1. *Dimensions of the spores of* Nosema branchiale ($\mu$)

| Measurements by | Length | | Width | | Length of polar capsule | | Length of polar filament |
|---|---|---|---|---|---|---|---|
| | Range | Mean | Range | Mean | Range | Mean | |
| Nemeczek, 1911 | — | 6·3 | — | 3·5 | — | — | *ca.* 90 |
| Dogiel, 1936 | 5·0–6·0 | — | — | — | — | — | — |
| Fantham *et al.* 1941 | 5·7–6·6 | — | 3·5–4·2 | — | — | — | 75–90 |
| Shulman & Shulman-Albova, 1953 | 4·3–5·1 | — | 2·0–2·3 | — | 2·3–2·6 | — | — |
| Kabata | 4·2–5·7 | 4·9 | 2·0–3·2 | 2·4 | 1·7–3·3 | 2·5 | — |

found in the pyloric caeca. Fantham, Porter & Richardson (1941) found a microsporidian, considered by them to be *N. branchiale*, on the gill filaments of cod examined in Montreal and probably caught off the Gaspé Coast, in the Gulf of St Lawrence. The parasite formed cysts, composed of fibrous reaction tissue, containing various stages of development. The cysts were 1 mm. in diameter. Shulman & Shulman-Albova (1953) found *N. branchiale*, during their survey of the parasite fauna of the White Sea fishes, infecting the White Sea shore cod (*Gadus morhua maris-albi* Derjugin). Three of sixty-eight examined fishes (4·4 %) were infected; one of the infected individuals was found to carry spores not only in the gill filaments but also in the tissues of the gut wall, in the liver, kidney, spleen, brain, spinal cord and vitreous humour.

The present author found *N. branchiale* on the gills of haddock caught in July 1957 off the south coast of Iceland, during the cruise of the research ship F.R.S. *Explorer*. The parasite formed easily noticeable whitish cysts, globular to oval in shape and 0·5–1·2 mm. in long diameter. The cysts were poorly defined in outline, appearing rather as swellings of the gill filaments; their longitudinal axes were always in alignment with those of the filaments. Over sixty cysts were found on one infected gill arch. At the time no attempt was made to examine the internal organs of the infected fishes. Unfortunately, also, no quantitative data of the occurrence of the parasite were collected. The gills of one infected individual were preserved, one part in formalin, another in Bouin's preservative, and brought back for examination. The cysts were examined in teased-out preparations, with Lugol's iodine and Gentian Violet stains, the latter being better for demonstration of the polar capsules. As fresh material was not available for examination, it was not possible to extrude and measure the polar filaments. (In all probability the same situation applied to Shulman's survey, as he does not give the length of the filament either.) Fifty spores were measured and the results are shown in Table 1.

Of the workers dealing with *N. branchiale* only Shulman & Shulman-Albova (1953) recorded any observations on its possible influence on the host fish. It appears that even the most seriously infected fish did not suffer any undue damage: at any rate, no harmful effects were indicated by the shape, coloration and the condition of the infected fish. The present author's observations are in agreement with these findings.

To investigate changes brought about by infection, the author examined a part of the gill arch, which was preserved in Bouin's fluid and prepared into sections, 4 $\mu$ thick, stained with Ehrlich's haematoxylin. It was found that the cysts of *N. branchiale* are formed exclusively in the connective tissue of the gill filament, surrounding the central skeletal rod. The position of the cyst in the filament is shown diagrammatically in Fig. 1, representing sections through infected and

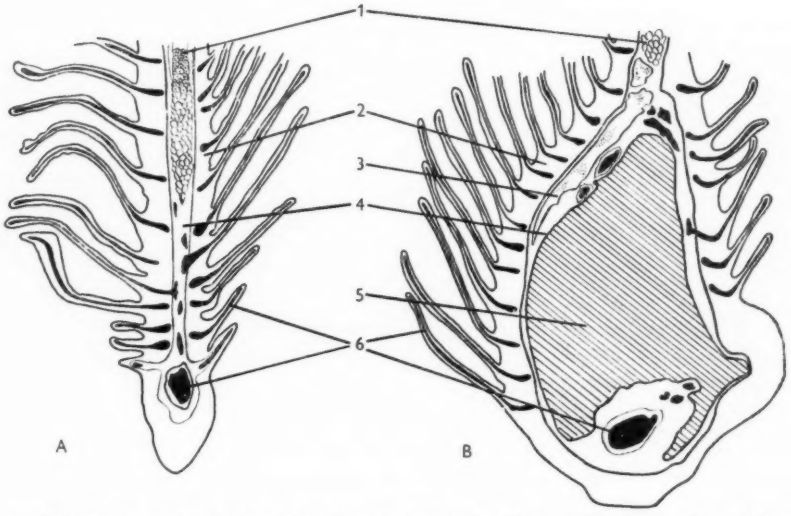


Fig. 1. Sections through gill filaments of haddock; (A) healthy gill filament; (B) gill filament infected with *Nosema branchiale*. 1, skeletal rod; 2, epithelial tissue; 3, section of empty cyst; 4, connective tissue; 5, the cyst; 6, blood vessels.

uninfected filaments. The plane of section is slightly oblique to the longitudinal axes of the filaments and passes at different levels through the supporting rod of cartilage, the connective tissue surrounding it and through one of the main blood vessels running longitudinally through that tissue. The cyst is formed exclusively in the connective tissue, which is fibrous in structure with fibres longitudinally arranged. When infected, this tissue becomes distended and disrupted by masses of spores, interspersed with partly destroyed cells and fibres. Such fibres within the cyst as are still recognizable are no longer longitudinally arranged. Spores are grouped in various numbers in small rounded lacunae throughout the affected tissue, and no other developmental stages are present. There seems to be no reaction tissue around the cyst in a well-defined capsule. At the edges of the cyst the affected connective tissue gradually gives place to healthy tissue; at no place

does the cyst abut directly on the blood vessels, filament epithelium or the cartilage rod. The sensitive respiratory epithelium, which is so prone to defensive hypertrophy, being always separated from the cyst by a layer of healthy tissue, remains completely unaffected: no changes can be observed in the structure of the respiratory folds overlying the infected area.

The cysts are far from being as regular in shape as may be observed externally: they push their way along the axes of the filaments, causing some disorganization in their structures. The skeletal rod may be somewhat displaced, and blood vessels are deflected from their normal courses, possibly also partially occluded and developing collateral branches. Some larger cysts are subdivided into several sections by lamina of healthy connective tissue. In some cases such sections, or the whole large cysts, appear to be almost completely empty, their only contents being small amounts of tissue debris, staining dark purple-black and showing no organized structure. It is possible that their infected contents are released by the rupture of the cyst wall to the exterior, but no traces of such ruptures could be found in the sections examined: the empty cysts showed no signs of collapse, retaining their shapes.

The apparent preference of the parasite for the connective tissue offers a possible clue to its extraordinarily wide dissemination in Shulman's material. It is possible that the parasite is specific to the connective tissue in general rather than to the gills. The usual microsporidian mode of infection (Kudo, 1924) is through the alimentary canal and the blood stream to the specific site. The presence of the cysts in the wall of the gut or in the pyloric caeca is therefore quite understandable. All the foci of infection quoted by Shulman appear to be located in the vicinity of abundant capillaries (liver, spleen, chorioid plexus, meninges). It is in such areas that the circulatory system offers the best possibilities for the parasite to break out and infect local connective tissue.

In summing up our present state of knowledge about *N. branchiale* it must be stated that the doubt, expressed by Kudo (1924), as to its position in the genus *Nosema* can no longer be entertained. The description by Fantham *et al.* (1941) of the schizogonic stages of development showed that only one spore is formed by each sporoblast and that the place of this parasite, therefore, is in the genus in which it was placed by its discoverer.

The records so far available of the occurrence of *N. branchiale* throw light on its geographical distribution. In the eastern North Atlantic no records have been obtained south of the latitude of 60°; the Canadian record comes from the latitude of about 48° N., but from the area which, climatically, does not differ greatly from that of the south coast of Iceland. *N. branchiale* was never observed by the author in the North Sea or Faroes, in spite of his having examined very large numbers of haddock and other gadoids for the presence of gill parasites. In view of these facts it can be assumed that *N. branchiale* is boreo-arctic in distribution. The work by various authors also shows dissimilarities in the characteristics of the parasite, particularly in the dimensions of the spores. They appear to fall into two size-groups, one being recorded by Nemeczek, Dogiel and Fantham, Porter & Richardson, and the other by Shulman & Shulman-Albova and Kabata (Table 1). The

method of cyst formation also seems to vary, the reaction tissue being present in the cysts described by Fantham, Porter & Richardson (1941), but absent from those examined by the present author. While there are many factors which can be responsible for the appearance of such differences, the possibility of the existence of more than one species in what has hitherto been described as *N. branchiale* should also be considered.

### (2) **Plistophora hippoglossoideos** Bosanquet, 1910

This parasite was described by its discoverer from material supplied to him by Dr Woodcock. The infected tissue was already embedded in paraffin and was studied in microscopical sections. The host fish was the long rough dab (*Drepanopsetta hippoglossoides* Gill) and the site of infection was given as 'fin-muscles', where the parasite formed small whitish nodules, oval in shape and from 1 to 2 mm. in diameter, which contained masses of pansporoblasts filled with ripe spores. These were oviform or piriform and, in view of their very small size, details of their internal structure were very difficult to observe, especially the size and the shape of the polar capsule. Several stages of sporulation were also found. The dimensions of the spores and the pansporoblasts are given in Table 2.

Table 2. *Dimensions of the spores of* Plistophora hippoglossoideos ($\mu$)

| | Bosanquet | Kabata | |
|---|---|---|---|
| | | Range | Mean |
| Diameter of pansporoblasts | 20–25 | 16–30 | 21 |
| Length of spores | 3·5 | 2·5–6·1 | 3·7 |
| Width of spores | 2·0 | 1·7–3·2 | 2·2 |
| Length of polar capsule (two measurements only) | — | 1·7 and 1·9 | |

Bosanquet did not know the locality in which the infected flatfish was found. Kudo (1924) gives it as England (with a query), but no other record of this parasite seems to exist in the literature. The present author has found *P. hippoglossoideos* to be widely distributed over the entire northern North Sea. In May 1958 alone long rough dab infected with this microsporidian were found in eleven localities (Fig. 2). The parasite thus does not appear to be unduly rare, and it is rather surprising that it has not been recorded more often.

It seems clear that the infections with *P. hippoglossoideos* can be much heavier than described by Bosanquet: one example of heavy infection is shown in Fig. 3. While the muscle tissue near the fins is sometimes infected, in the majority of cases the infection involves primarily the walls of the visceral cavity, both on the eyed and the blind sides. It will be seen from Fig. 3 that the cysts formed by *P. hippoglossoideos* cannot be referred to as 'small nodules': they are oblong structures with rounded edges, closely packed, sometimes in quite large numbers and reaching the size of 10 mm. in length and 2·5 mm. in short diameter.

The spores of *P. hippoglossoideos* were examined by the author in teased-out preparations, stained with Lugol's iodine and Gentian Violet: fifty spores were

measured and the measurements are shown in Table 2. The small size of the spores and the fact that, unlike those of *N. branchiale*, they did not take up stains easily, made observations of their internal structure very difficult. The best results were obtained by observing unstained spores, using the interference microscope, with $\times 800$ magnification. The spores were very irregular in shape and variable in size. Besides spores which were almost round ($3{\cdot}0 \times 2{\cdot}5\ \mu$ and $2{\cdot}8 \times 2{\cdot}3\ \mu$), others of greatly elongated shape ($6{\cdot}1 \times 2{\cdot}2\ \mu$ and $6{\cdot}1 \times 2{\cdot}3\ \mu$) were present. Some were

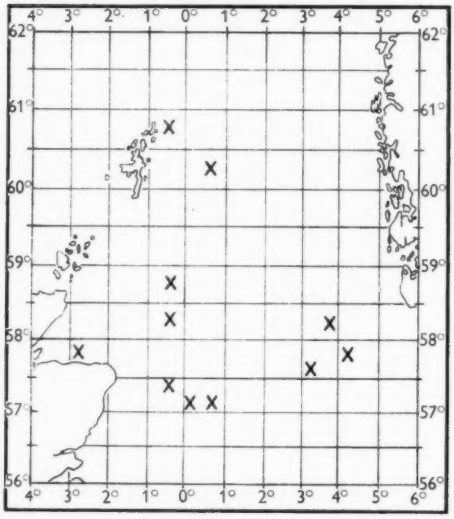


Fig. 2

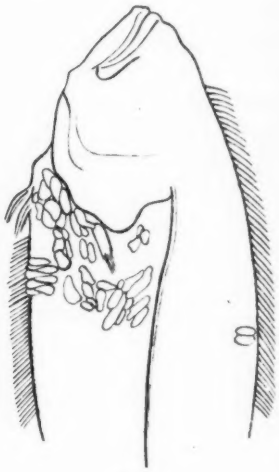

Fig. 3

Fig. 2. Localities (marked ×) in the northern North Sea, where *Plistophora hippoglossoideos* was found in May 1958.

Fig. 3. Blind side of long rough dab, showing nodules formed by *Plistophora hippoglossoideos* (about natural size).

bent in the shape of a fat sausage, while in one case a fusion of such bent spores by their convex surfaces was observed. Only three of the spores measured had their polar capsules distinct enough to allow measurements to be taken.

The dimensions ($\mu$) of those spores were as follows:

| Length | Width | Length of polar capsule |
|---|---|---|
| 3·0 | 2·0 | 1·7 |
| 4·2 | 2·2 | 1·9 |
| 6·1 | 2·2 | 3·8 |

In each case the length of the polar capsule corresponded roughly to a half of the total length of the spore. This does not agree with Bosanquet's illustration, which shows the polar capsule as being about a quarter to one-third of the length of the spore. Examination by means of the interference microscope showed in several spores the presence of a vacuole, which was mentioned by Bosanquet as

being present in the sporoplasm, but no trace was found of a 'central dot', seen by him in the centre of the vacuole.

Some of the nodules were prepared by standard methods into sections, 4 $\mu$ thick, which were examined after staining with Ehrlich's haematoxylin. The nodules are formed in the body wall with considerable displacement of the muscle; usually they are found to project somewhat into the body cavity, from which they are separated only by peritoneum. Parts of the muscle are imprisoned and compressed between the adjacent nodules. The latter are filled, as Bosanquet observed, with masses of pansporoblasts, containing almost exclusively mature spores in the centres of the cysts, but, nearer the edge, sometimes the earlier stages of sporulation.

Bosanquet stated that no reaction of the host tissue could be observed. This is fully confirmed by the present observations: no cellular infiltration could be found and no destruction of the host tissues; even the parts of muscle compressed between the cysts appeared histologically quite normal. It seems, then, that no great damage is suffered by the host as the result of infection with this protozoan.

It is hoped that these brief notes may serve as a reminder of the great scope and interest which is to be found in the study of the parasite fauna of the marine fishes.

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# *DENDROGASTER TASMANIENSIS* SP.NOV. (ASCOTHORACIDA) FROM THE SEA-STAR *ALLOSTICHASTER POLYPLAX* (MÜLLER AND TROSCHEL)

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(With 19 Figures in the Text)

## INTRODUCTION

The Ascothoracida recorded from sea-stars have been placed either in the genus *Dendrogaster* Knipowitsch, 1891 or in the genus *Myriocladus* Okada, 1925.

In 1946, Wagin considered that the genus *Dendrogaster* should contain only the three species: *D. astericola* Knipowitsch, 1891; *D. arctica* (Fisher, 1930) Korschelt, 1933 and *D. murmanensis* Kluge n. nud. Korschelt, 1933; and that the genus *Myriocladus* should include the following five species: *M. okadai* (Okada, 1925) Yosii, 1931; *M. astropectinis* Yosii, 1931; *M. arborescens* (Le Roi, 1905) Okada, 1925; *M. ludwigi* (Le Roi, 1905) Okada, 1925; and *M. arbusculus* (Fisher, 1911) Okada, 1925. Later in the same year Vaghin (an incorrect spelling of Wagin) transferred *M. astropectinis* and *M. arborescens* to the genus *Dendrogaster* and added two new species, namely, *D. rimsky-korsakovi* and *D. leptasteriae*. Unfortunately he omitted to name the hosts of the two new species.

At least five of the ten species mentioned above are insufficiently described. However, the external form of the female is known in each case and is fundamentally the same in all ten species. Thus all the females agree in that externally each is composed of a cylindrical or conical middle piece, from the posterior end of which is given off, on each side, a branched outgrowth of the mantle. The degree of branching varies not only from species to species but also within a single species. All known males, likewise, show a basic similarity in form. Each has a well-segmented body enclosed in a bivalved mantle, each valve forming a posterior sac-like outgrowth.

The females of all the species occur free in the body cavities of their respective hosts. All the males that have been discovered have been found in the mantle cavities of the females. Lastly, all the hosts are members of the Asteroidea. In view of these similarities, the ten species appear to be congeneric and in my opinion *Myriocladus* Okada is a synonym of *Dendrogaster* Knipowitsch. The genus *Dendrogaster* may therefore be defined as follows: Ascothoracids of the family Dendrogasteridae, in which the female consists of a middle piece having on each side at the posterior end a branched outgrowth of the mantle, the outgrowths containing extensions of the gut, gonad and mantle cavity. Sexual dimorphism is marked, the male being much smaller than the female and having a well-segmented body enclosed within a bivalved mantle. Each valve forms a posterior sac-like outgrowth containing a testis and a diverticulum of the gut. The eggs

develop in the mantle cavity of the female and there hatch at the nauplius, metanauplius or ascothoracid stage; the larvae escape from the mantle cavity in the ascothoracid stage. The females are found unattached as endoparasites in the body cavity of various Asteroidea; the males inhabit the mantle cavity of the females. Type of the genus, *D. astericola* Knipowitsch, 1891.

*Dendrogaster tasmaniensis* sp.nov. described in the present paper occurs in the body cavity of the asteroid *Allostichaster polyplax* (Müller and Troschel) and is the first species of the genus to be recorded from the South Pacific.

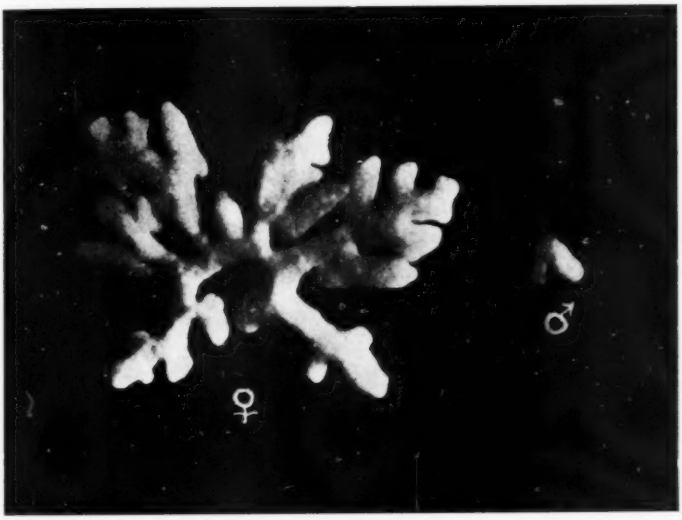



Fig. 1A. Mature female and male of *Dendrogaster tasmaniensis* sp.nov.

## MATERIAL AND METHODS

One hundred and sixteen specimens of the host *A. polyplax* were collected from the following localities in Tasmania: Ralph's Bay, Sandy Bay, Blackman's Bay, Adventure Bay and Eaglehawk Neck. Those from Ralph's Bay and Sandy Bay were obtained by dredging at 6–8 fathoms. The specimens from other localities were collected from under stones in the intertidal zone.

From the body cavities of twenty-six of the sea-stars collected, a total number of thirty-six females of *D. tasmaniensis* sp.nov. was obtained. In both the body cavity and the stomach of one specimen of *A. polyplax* numerous larvae were found. The dissection of six of the female parasites yielded a total of eighteen males. In a whole mount of another female, two more males were found.

The larvae and some of the females were fixed in Bouin's fluid. Other females were fixed in Gilson's fluid. After fixation some larvae were dissected by means of fine needles, made by drawing out glass rod in a Bunsen flame. The separate parts of the larvae were then mounted unstained in lactophenol. Other larvae were stained in alum carmine. Several of these were mounted whole in Canada balsam

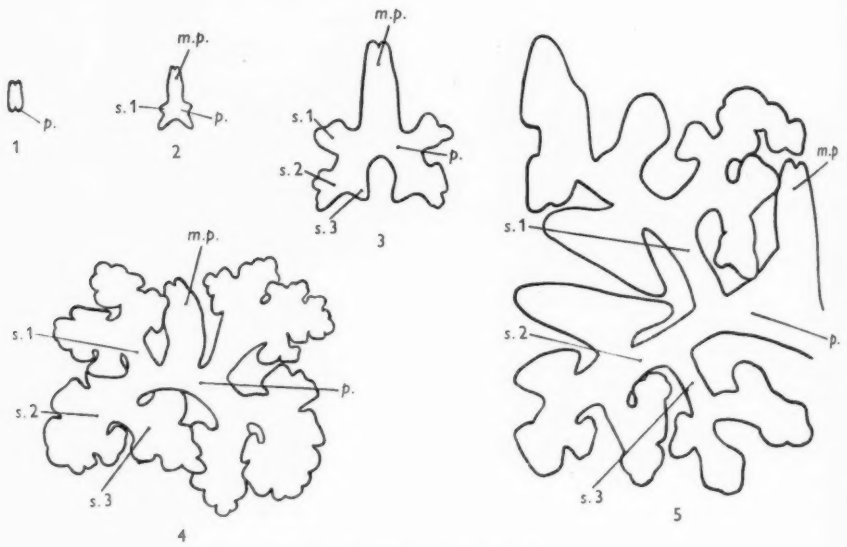
and some were dissected before mounting. A few larvae, two females and three males, were boiled in 10% sodium hydroxide until they were clear. They were then rinsed in 50% acetic acid followed by glacial acetic acid, stained in acid fuchsin (0·2 g. in 100 c.c. glacial acetic acid) for 12–24 hr. and transferred to slides. After rinsing with glacial acetic acid to remove the excess of stain the females were mounted whole in euparal. The larvae and the males were dissected before rinsing and mounting. Acid fuchsin proved very satisfactory for staining chitinous parts.

Serial sections of an immature female, four mature females, a mature male and several larvae were cut at 10$\mu$ and stained in Ehrlich's haematoxylin.

## DESCRIPTION

### *Female* (Figs. 1–10)

Colour, white or cream, with a pair of median dorsal longitudinal orange streaks on the posterior half of the middle piece. Postero-laterally, the streaks are continued out to the extremities of the branches.




*Dendrogaster tasmaniensis*

Fig. 1. Dorsal view of the smallest female found in the body cavity of the host. Figs. 2–4. Ventral views of immature females, illustrating the development of the branching of the mantle. Fig. 5. Dorsal view of the mature female (left half only), showing a short posterior secondary branch, S3.

Middle piece, elongate and cylindrical, with a conical anterior end, which has a short dorso-ventral slit-like opening leading into the mantle cavity.

Two primary branches, one on each side, arising from the posterior of the middle piece, pass outwards and backwards. Each forms three secondary lateral branches.

There are thus anterior, middle and posterior pairs of secondary lateral branches. The mean length of the anterior pair is the greatest and that of the posterior pair the smallest. In some specimens the posterior pair is so short that there appear to be only two pairs of branches (Fig. 5). Arising in an alternate manner on either side of the secondary branches are 4–8 tertiary branches, each of which may be lobe-like or may bear 1–4 quaternary lateral branches. These quaternary branches

Table 1. *External measurements (in mm.) of thirteen females of* Dendrogaster tasmaniensis *sp.nov.*

| Width of specimen | Middle piece | | Mean length of secondary branches | | | Maturity of specimen |
|---|---|---|---|---|---|---|
| | Length | Width | S1 | S2 | S3 | |
| 0·42 | 0·70 | 0·42 | — | — | — | Immature |
| 0·84 | 0·91 | 0·49 | 0·07 | — | — | Immature |
| 0·92 | 0·91 | 0·53 | 0·07 | — | — | Immature |
| 6·30 | 3·57 | 1·26 | 2·45 | 1·93 | 1·57 | Immature |
| 7·49 | 2·10 | 1·05 | 2·97 | 1·99 | 1·65 | Immature |
| 8·50 | 3·57 | 1·12 | 2·98 | 2·35 | 1·54 | Immature |
| 9·20 | 2·80 | 1·19 | 2·98 | 2·80 | 1·65 | Immature |
| 9·31 | 2·10 | 1·40 | 3·92 | 3·05 | 2·03 | Mature; without larvae |
| 11·00 | 2·66 | 1·19 | 4·59 | 2·98 | 1·96 | Mature; without larvae |
| 14·70 | 1·82 | 1·19 | 6·05 | 5·88 | 5·08 | Mature; without larvae (Type) |
| 15·40 | 2·10 | 1·75 | 7·35 | 6·28 | 4·60 | Mature; with larvae |
| 16·90 | 7·35 | 1·82 | 8·40 | 5·60 | 4·55 | Mature; with larvae |
| 18·90 | 3·50 | 1·40 | 10·15 | 5·25 | 5·53 | Mature; with larvae |

are usually lobe-like and a few may bear 1–3 short lobe-like lateral branches of the fifth order. Only one of the eight mature females in the collection appears to have a different mode of branching of the mantle. In this specimen each secondary branch divides dichotomously (Fig. 7). The ends of all branches are bluntly rounded.

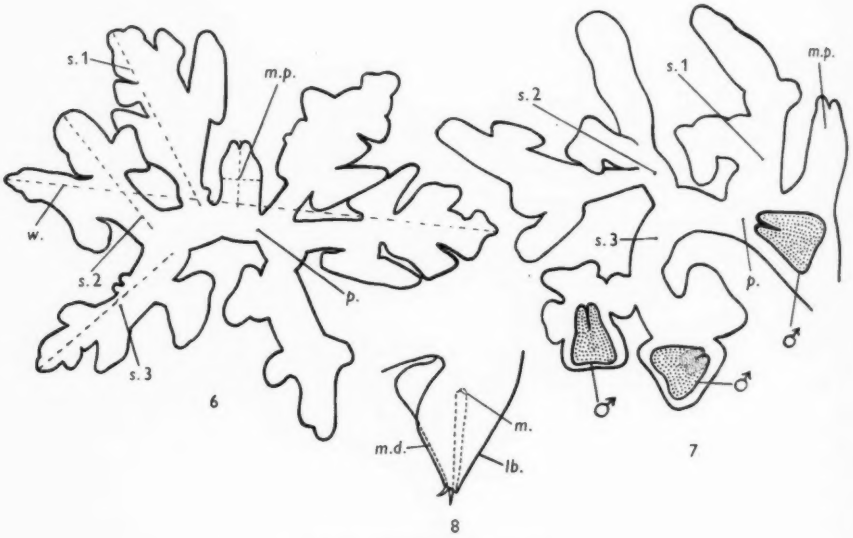
In Table 1, external measurements of thirteen females are listed, and in Fig. 6 the axes along which these measurements have been made are shown.

The sides of the slit-like opening of the mantle cavity are beset with numerous small projections. The mantle cavity, gut and gonad extend from the middle piece out into the branches.

The body within the mantle cavity of the middle piece lacks external segmentation. A separate cephalothorax and abdomen are not distinguishable. Anteriorly the body bears the antennules, antennae and oral cone.

The antennules (Figs. 9, 10) arise from the sides of the anterior part of the body within the mantle cavity. They are about 0·48 mm. long. In life their distal end can be protruded through the opening of the mantle cavity. They are laterally compressed and each has six segments. Viewed laterally, the first or proximal segment appears nearly triangular. The apex is directed upwards and forwards. Its dorso-ventral diameter is about twice the length of the segment. Dorsally it is membranous and the inner face is less chitinized than the outer. The second

segment, which is about as long as the first, has a dorso-ventral diameter equal to three-quarters that of the first segment. Except for a proximo-dorsal transverse chitinous bar, the dorsal surface of the segment is membranous. Its sides and base are completely chitinized. Distally it articulates with both the third and fourth segments. The third segment, which is half the length of the second, is situated dorsal to the fourth. It is thus incomplete ventrally. Viewed from the outer side it is nearly triangular in shape. The apex, which is blunt, is directed forwards and bears a pair of stout movable spines, which measure 0·016 mm. long and point towards the front. The proximal end of the segment has a dorso-ventral diameter equal to about three-quarters the length of the segment. The fourth segment, which articulates proximally with the second, dorsally with the third and distally




*Dendrogaster tasmaniensis*

Fig. 6. Ventral view of the mature female (type specimen), showing a long posterior secondary branch, S 3. Broken lines indicate the axes along which the measurements given in Table I were taken. Fig. 7. Dorsal view of the mature female (left half only), showing the dichotomous branching of the secondary branches, S 1, S 2 and S 3. Three males are present in the mantle cavity; the two in which valves are not visible are inverted. Fig. 8. Lateral view of the oral cone of the female.

with the fifth, has a dorso-ventral diameter equal to its length and about three-quarters the dorso-ventral diameter of the second segment. Dorsally, where it articulates with the third segment, it is incomplete. The length of the fifth segment is about half its dorso-ventral diameter and less than half the length of the fourth segment. Its dorsal surface is conical. Arising from the upwardly directed apex is a strong recurved movable claw. Two spines similar to those on the third segment occur near the base of the claw; one arises behind the base and projects upwards, the other is situated on the inner face a short distance below the front

of the base and projects forwards. The outer antero-ventral face of the segment is membranous and bears a spine in addition to a short blunt process. The spine, which is similar to the other spines on the segment, is situated anterior to the process. The latter is the remains of the olfactory filament which, in the larvae and males, is well developed. The sixth segment is conical and has its apex directed upwards. Its length is about twice its proximal dorso-ventral diameter. It bears three spines similar to those on the third and fourth segments. Two arise from the apex and the third is on the anterior face a short distance below the apex.

The antennae, situated laterally, are postero-ventral to the bases of the antennules and appear to resemble those of the male. Unfortunately, a satisfactory mount of a complete female antenna was not obtained.

The oral cone is formed by the labrum, which extends downwards beyond the mouth anteriorly and laterally. The lateral extensions pass round behind the mouth-parts, so that the labrum forms a hollow conical structure, the apex of which is open and directed downwards. Behind the mouth-parts these extensions interlock but do not fuse. A chitinous thickening is present on the anterior inner face of the oral cone and extends from the apex of the cone up to and around the mouth. The chitin along the posterior juncture of the labrum with the body forms a thickening on each side. These thickenings extend from the mouth to the dorso-lateral limits of the labrum, where they pass internally as a pair of curved hollow rods. These rods pass upwards, curving inwards and forwards in front of the transverse adductor muscle of the mantle and behind the brain. Here they are situated one on either side of the nerve cord. Although these rods are hollow, their dorsal ends, which are near the antero-dorsal extremity of the gut, are closed. They serve for the attachment of muscles operating the oral cone.

Projecting down from behind the mouth and surrounded by the labrum is a pair of subulate mouth parts. Since these are situated immediately behind the mouth and evidence of other mouth parts is lacking, they are referred to as mandibles. From each mandible a short distance from its pointed end, there arises posteriorly a movable hook-like process. The length of this process is equal to the length of that part of the mandible distal to the point of articulation of the process. When the mandible is retracted within the oral cone the process fits into a groove on the posterior face of the distal part of the mandible. When the end of the mandible is protruded out of the oral cone, the process can be flexed back at right angles to the long axis of the mandible.

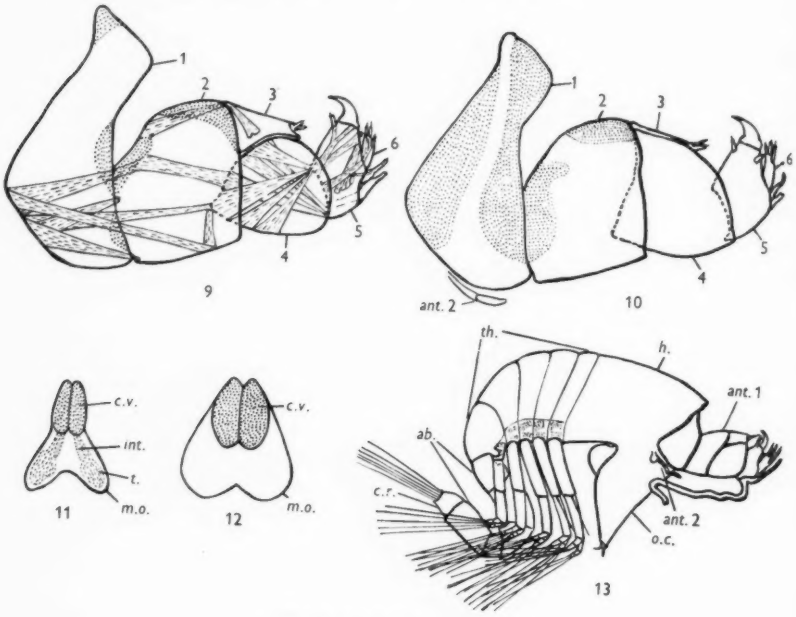
Mature females occur within the disk of the sea-star and close beneath the aboral surface. In large specimens the branches of the mantle extend into the arms of the asteroid. Immature females are found either in the disk or in the arms of the host.

*Male* (Figs. 11–18)

Males are white or cream coloured and when mature are 1·1–2·1 mm. long. Viewed from above, young males are shaped like an inverted Y; mature and old males are somewhat triangular or oval (Figs. 11, 12). The mantle forms two lateral valves enclosing the rest of the animal. The outer surface of each valve is heavily chitinized and is 0·87 × 0·60 mm.; the inner surface is membranous and forms a

blunt unbranched posterior outgrowth extending about 1·0 mm. beyond the chitinous part. Each outgrowth contains a testis and a diverticulum of the gut. In mature and old specimens, the membranous part of the mantle becomes so inflated that the chitinous outer part comes to lie dorsally.

The body between the valves is laterally compressed and externally segmented. A head, thorax and abdomen are distinguishable (Fig. 13).



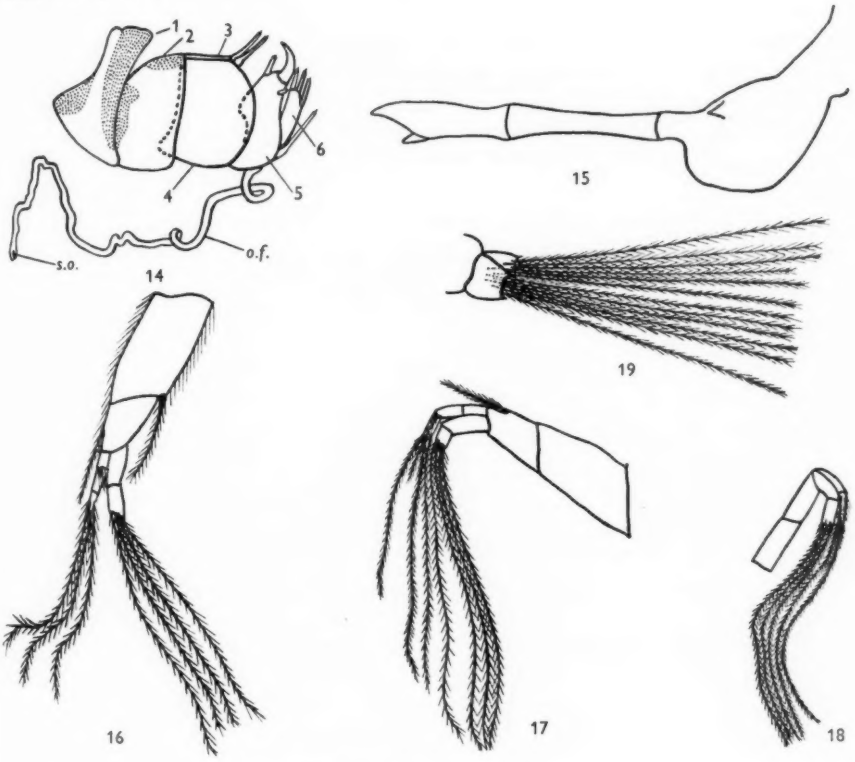

*Dendrogaster tasmaniensis*

Fig. 9. Right antennule of the female. (Membranous parts are stippled.) Fig. 10. Left antennule and part of the antenna of the female. (Membranous parts are stippled.) Fig. 11. Dorsal view of the young male. Fig. 12. Dorsal view of the mature male. Fig. 13. Lateral view of the mature male with the mantle removed. (Thoracic pleurites are stippled.)

The head bears anteriorly the antennules and antennae. The former differ from those of the female in that they are smaller (0·28 mm. long) and possess an olfactory filament and longer spines (Fig. 14). The antennae are situated laterally, postero-ventral to the bases of the antennules. They are small and inconspicuous. Each is approximately 0·16 mm. long and consists of four segments 0·08, 0·046, 0·041, 0·005 mm. long. The proximal segment is more heavily chitinized than the others and is swollen ventrally. It bears dorsally, on the post-axial or outer side of the distal third, a short tooth-like spine. The distal end of the third segment extends some distance beyond the articulation with the very small fourth segment. Setae are absent. Projecting from beneath the head is the oral cone, which encloses a pair of mandibles similar in structure to those of the female.

There are five distinct segments in the thorax. Each bears a pair of natatory appendages. Proceeding posteriorly, the segments increase in length and decrease

in dorso-ventral diameter. The thoracic tergites are strongly convex dorsally. On each side the ventral margin of the fifth tergite is conical. In each segment, between the coxopodites of the appendages and the ventral margins of the tergites, are two pleurites. One is dorsal to the other, and in segments 1–4 it is the larger. In segment 5 the ventral pleurite is the larger. The sternites are narrow, transverse, strap-like plates.




*Dendrogaster tasmaniensis*

Fig. 14. Left antennule of the male. (Membranous parts stippled.) Fig. 15. Left antenna of the male. Fig. 16. First right thoracic appendage of the male. Fig. 17. Third right thoracic appendage of the male. Fig. 18. Fifth left thoracic appendage of the male. Fig. 19. Lateral view of the caudal rami of the male.

Each thoracic appendage is biramous and consists of a protopodite of two segments, an exopodite of two segments and, in all except the posterior pair of appendages, a three-segmented endopodite. The endopodite of the last pair is composed of two distinct segments. The first and second pairs of appendages are similar. The coxopodite and inner side of the basipodite have a dense covering of fine setae. On the outer side of the distal end of the coxopodite is a long plumose seta. A similar but shorter seta is present on the inner side of the distal half of the basipodite. The terminal segment of the exopodite bears distally four very

long plumose setae. On the outer side of the distal end of the proximal segment of the endopodite is a tuft of setae. The distal segment bears three long plumose setae, of which two are terminal and one is subterminal. The third and fourth pairs of appendages are similar to each other. They differ from the first and second pairs of appendages in possessing a short plumose seta on the inner side of the distal end of the second segment of the endopodite and in lacking the covering of fine setae on the protopodite, the plumose seta on the coxopodite and the tuft of setae on the proximal segment of the endopodite. The protopodite of the fifth appendage has no covering of fine setae and both the coxopodite and the basipodite lack plumose setae. There are only three long plumose setae on the distal end of the exopodite. As noted previously, the endopodite has only two segments. The second segment has a covering of fine setae and at its distal end bears two long plumose setae.

The abdomen is composed of five segments. Measured in order from anterior to posterior along the dorsal margin, the segments have the following lengths (in mm.): 0·085, 0·082, 0·028, 0·023, 0·099. The lengths (in mm.) along the ventral margin are as follows: 0·08, 0·138, 0·08, 0·035, 0·115. The first segment bears mid-ventrally the forwardly projecting conical penis. Articulating with the distal end of the fifth segment are two short caudal rami. At the distal end of each ramus are two short spines, one dorsal, the other ventral. The dorsal spine is the longer. Arising from the inner side of each ramus are six long backwardly projecting plumose setae.

All the males examined were found in the mantle cavity of females. Their number and distribution are shown in Table 2. Young and sexually mature males lie with their dorsal and ventral surfaces corresponding with those of the female, whilst older males, which have shed their sperms, are inverted.

Table 2. *Distribution and maturity of males in the mantle cavity of females*

| Date collected | Maturity of female | No. and maturity of males | Position of males in the mantle cavity of female |
|---|---|---|---|
| 29. viii. 55 | Mature; without larvae | 2 mature | End of secondary branches |
| 3. xi. 55 | Mature; with larvae | 2 young, 2 mature | End of quaternary branches |
| 14. ii. 57 | Immature | No males present | — |
| 14. ii. 57 | Mature: without larvae | 1 young, 2 mature | End of tertiary branches |
| 14. ii. 57 | Mature; with larvae | 4 mature, 1 old | End of quaternary branches |
| 14. ii. 57 | Mature; with larvae | 1 mature, 2 old | One old specimen in the posterior of middle piece; rest at end of quaternary branches |
| 28. xi. 57 | Mature; without larvae | 1 mature | End of secondary branch |
| 28. xi. 57 | Mature; with larvae | 2 mature | One in secondary branch, other at end of quaternary branch |

*Larva*

The larvae hatch in and are liberated from the mantle cavity of the females as typical cypris-like ascothoracid larvae. No sexual differentiation between the larvae was observed. Externally they differ from the adult males only in having slightly shorter mantle valves and in lacking the inflated outgrowths of the mantle.

*Type.* In the Department of Zoology, University of Tasmania.

*Host. Allostichaster polyplax* (Müller and Troschel).

*Habitat.* Body cavity.

*Localities.* Sandy Bay, Ralph's Bay, Blackman's Bay and Adventure Bay, Tasmania.

## AFFINITIES OF *DENDROGASTER TASMANIENSIS* SP.NOV.

As mentioned in the introduction, of the ten previously recorded species belonging to the genus *Dendrogaster*, at least five have been inadequately described, thus making comparisons difficult. However, the general form of the female is known in all species and that of the male in nine species. The males of *D. tasmaniensis* sp.nov. resemble those of *D. astericola* and *D. murmanensis* and differ from those of other species in having the posterior outgrowth of the mantle very short. Moreover, the males of the Tasmanian species are of a much smaller average size than are those of the other species. *D. tasmaniensis* sp.nov. may be distinguished from *D. astericola* in its female having a much greater degree of branching. It differs from *D. murmanensis* in having females of a markedly smaller size and in the females containing fewer males. In the case of the Californian species, *D. arbusculus*, the female has been figured, but not described. Nothing is known of its male. The female of *D. tasmaniensis* sp.nov. differs from *D. arbusculus* in lacking a posterior median outgrowth of the mantle and in having a smaller degree of branching.

## INFESTATION OF THE HOST

Infested individuals were found, not only among the sea-stars collected in the intertidal zone, but also among those obtained from a depth of 6–8 fathoms.

The presence of the parasite causes an aboral swelling of the disk of the sea-star and the displacement of the internal organs. No other effect of the parasite on the host was observed. The degree of infestation is shown in Table 3.

Of the 116 sea-stars examined, 97 were measured. The size in relationship to the frequency of infestation is shown in Table 4. The table reveals a remarkable connexion between the size of the sea-star and the presence or absence of the parasite. Thus, of the ninety-seven asteroids measured and examined, forty-one had a disk diameter of 11 mm. or less and were all non-infested; of the fifty-six with disk diameter of 12 mm. or more, seventeen were infested. A possible explanation of this relationship is presented later in this work. The smallest *Allostichaster polyplax* infested with a female *Dendrogaster tasmaniensis* sp.nov. containing active larvae, had a disk diameter of 15 mm.

Table 3. *The infestation of* Allostichaster polyplax (*Müller and Troschel*) *with* Dendrogaster tasmaniensis *sp.nov.*

| Locality and date collected | No. of *A. polyplax* Examined | Infested with 1 parasite | Infested with 2 parasites | Infested with 3 parasites | Total no. of *A. polyplax* infested | Total no. of *D. tasmaniensis* found |
|---|---|---|---|---|---|---|
| Ralph's Bay, 24, 29. viii. 55 | 8 | 1 | 0 | 1 | 2 | 4 |
| Sandy Bay, 23. ix. 55 | 8 | 2 | 1 | 1 | 4 | 7 |
| Eaglehawk Neck, 31. x. 55 | 19 | 0 | 0 | 0 | 0 | 0 |
| Blackman's Bay, 3. xi. 55 | 17 | 3 | 0 | 0 | 3 | 3 |
| Blackman's Bay, 14. iii. 57 | 3 | 0 | 0 | 1 | 1 | 3 |
| Adventure Bay, 14. ii. 57 | 28 | 4 | 0 | 0 | 4 | 4 |
| Blackman's Bay, 28. xi. 57 | 24 | 5 | 1 | 0 | 6 | 7 |
| Blackman's Bay, 30. xi. 57 | 9 | 4 | 2 | 0 | 6 | 8 |
| Total | 116 | 19 | 4 | 3 | 26 | 36 |

Table 4. *Size of* Allostichaster polyplax (*Müller and Troschel*) *in relationship to frequency of infestation by* Dendrogaster tasmaniensis *sp.nov.*

| *A. polyplax* Diameter of disk (mm.) | No. examined | No. infested | *A. polyplax* Diameter of disk (mm.) | No. examined | No. infested |
|---|---|---|---|---|---|
| 4 | 1 | 0 | 12 | 18 | 2 |
| 5 | 1 | 0 | 13 | 12 | 3 |
| 6 | 3 | 0 | 14 | 12 | 4 |
| 7 | 6 | 0 | 15 | 6 | 3 |
| 8 | 4 | 0 | 16 | 3 | 2 |
| 9 | 3 | 0 | 17 | 2 | 1 |
| 10 | 11 | 0 | 18 | 3 | 2 |
| 11 | 12 | 0 | Total | 97 | 17 |

*The manner in which the larvae escape from the host*

A number of larvae were observed swimming out from under a sea-star. On dissection, the asteroid was found infested with a large female *D. tasmaniensis* sp.nov., the mantle cavity of which contained active larvae. Both the body cavity and stomach of the sea-star also contained numerous larvae. Exuviae of the larvae were found in the mantle cavity of the parent and the body cavity of the host.

It appears that, on hatching, the larva moults at least once in the mantle cavity of the female. Then it bursts the mantle and enters the body cavity of the host, where it undergoes at least one further moult, after which it penetrates the host's stomach and escapes to the exterior through the mouth of the sea-star.

*The manner in which the larvae enter the host*

Three live sea-stars (A, B, C) were placed in separate Petri dishes containing fresh sea water and a number of larvae which had escaped from the ruptured mantle cavity of a female *D. tasmaniensis* sp.nov. Some larvae, after swimming around in a typical jerky fashion, attached themselves, by means of their antennules and mouth parts, to the aboral dermal branchiae of the sea-star. After an hour, two larvae were found trying to enter sea-star A through the dermal pores. Each had collapsed a branchia and one was nearly half in a pore. A third larva was found inside a dermal branchia of asteroid C. Sea-stars A and B were examined at the end of 2 days and C after 5 days. A, which had been exposed experimentally to two larvae, contained one larva free in the body cavity of an arm; B, exposed to eight larvae, contained four larvae, two of which were attached to a pyloric caecum; C, exposed to thirteen larvae, contained seven larvae and one immature female.

Though the asteroid C may have become infested prior to the experiment, it seems unlikely that all three sea-stars had become infested naturally just before the experiment.

Although the complete entry of a larva was not observed, the available evidence suggests that the larva gains an entrance into the host through the dermal pores. If, as might be expected, the size of the pores increases with the size of the sea-star, then pore size in relation to the size of the larva may account for the absence of infestation in asteroids with a disc diameter of 11 mm. or less.

*The significance of exuviae found in the mantle cavity of a female*

Two mature males and four exuviae were found in the mantle cavity of a mature female which had been mounted whole after sodium hydroxide-acid fuchsin treatment. The exuviae consist of antennules, oral cone and mandibles. The absence of valves, thoracic and abdominal exoskeleton, excludes the possibility of the exuviae being from larvae or males. In the female most of the exoskeleton is membraneous. Only that of the antennules, oral cone and mandibles is chitinous. Since these parts are enclosed in the mantle cavity, their exoskeleton, when cast, could be retained in this cavity. The presence of these four female exuviae suggests that, once it is inside the host, the female undergoes at least four moults.

Apart from the occurrence of mature males in the female mantle cavity, little is known of the life of the males. A parthenogenetic origin for males of the Dendrogasteridae has been suggested (Wagin, 1946, pp. 238–9). If the two males had developed parthenogenetically from eggs of the female, then exuviae of the developmental stages of the males would also have been present in the mantle cavity. Their absence suggests that such an origin is unlikely.

## DISCUSSION

Pyefinch (1936, pp. 656–8), in his account of *Baccalaureus hexapus*, describes appendages which he suggests might be antennae. However, Krüger (1940), Wagin (1946), and in particular Brattström (1956), are of the opinion that the

adult ascothoracid, like the adult cirripede, lacks antennae. The nauplii and metanauplii of the Ascothoracida possess antennae and according to Brattström (1948) they are present in the early ascothoracid (cypris) stage of *Ulophysema öresundense*. The discovery of appendages in the female, male and ascothoracid larvae of *Dendrogaster tasmaniensis* sp.nov., which from their form and position are interpreted as antennae, is therefore of considerable interest. They appear to be homologous with: (*a*) the 'Auswuchse' of the larvae of *D. astericola*—Knipowitsch (1892, p. 137); (*b*) the 'peculiar organ' of *Synagoga mira*—Norman (1913, p. 164); (*c*) the 'appendice dont la fonction est incertaine' or 'appendices particuliers' of the larva of *Myriocladus* and larva and adults of *Synagoga* species—Okada (1926, p. 70; 1939, pp. 492, 494, 510); and (*d*) the antennae of the larvae of *Ulophysema öresundense*—Brattström (1948, pp. 36, 38, 64). In each case the appendage is attached in a similar position. Okada (1939) states that in *Synagoga* species the appendage is better developed in males than in females. Wagin (1946) draws attention to the affinities between the Ascothoracida and the Copepoda. That the adults of some Ascothoracida possess antennae is further evidence of this affinity.

Little is known about the life cycle of the Dendrogasteridae. Unlike the larvae of *D. murmanensis*, which penetrate into their host through the germinal pore (Wagin, 1946, p. 205), the larvae of *D. tasmaniensis* sp.nov. enter their host through the dermal pores. A parthenogenetic origin for the males in the Dendrogasteridae has been suggested (Wagin, 1946, p. 238), but as mentioned previously, observations on *Dendrogaster tasmaniensis* sp.nov. do not support such a view. When and how the male enters the mantle cavity of the female is still unknown.

## SUMMARY

The genus *Myriocladus* Okada is considered to be a synonym of the genus *Dendrogaster* Knipowitsch.

The female and male of a new species, *D. tasmaniensis* sp.nov. from the sea-star *Allostichaster polyplax* (Müller and Troschel), are described.

Appendages interpreted as antennae are present in the female, male and ascothoracid larva of the new species.

A brief account is given of the manner in which the larva of *D. tasmaniensis* sp.nov. enters and escapes from the host.

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## KEY TO LETTERING OF FIGURES

| | | | |
|---|---|---|---|
| *ab.* | abdomen | *m.o.* | mantle outgrowth |
| *ant.*1 | antennule | *m.p.* | middle piece |
| *ant.*2 | antenna | *o.c.* | oral cone |
| *c.r.* | caudal ramus | *o.f.* | olfactory filament |
| *c.v.* | chitinous valve | *p.* | primary branch |
| *h.* | head | *s.*1–3 | secondary branches |
| *int.* | intestine | *s.o.* | sensory organ |
| *lb.* | labrum | *t.* | testis |
| *m.* | mouth | *th.* | thorax |
| *md.* | mandible | *w.* | axis of maximum width |

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# THE LIFE HISTORY AND DEVELOPMENT OF *TOXASCARIS LEONINA* (von LINSTOW 1902) IN THE DOG AND CAT

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(With Plates VI and VII and 40 Figures in the Text)

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## I. INTRODUCTION

### (*a*) *Nomenclature and hosts*

Rudolphi (1809) described an ascaridoid nematode from the intestine of a lion and named it *Ascaris leptoptera*. It is quite clear from his description that it possessed narrow cervical alae. In so far as we now know that the lion harbours two-arrow-headed species, one with broad cervical alae (*Toxocara cati*) and the other with narrow cervical alae (*Toxascaris leonina*), it is probable that *Ascaris leptoptera* Rudolphi, 1809 was the same as the latter species, which was named and described by von Linstow (1902) from the same host. Rudolphi (1819) reported on some further specimens of *A. leptoptera* from a lion, but it appears from Bremser (1824) and Schneider (1866) that *A. leptoptera* Rudolphi 1819 may have been *Toxocara cati*.

In view of this uncertainty, it seems preferable to retain von Linstow's species, i.e. *Ascaris leonina*, rather than resurrect *A. leptoptera* for the narrow-winged

ascaridoid of the lion, although the latter was used throughout the nineteenth century and was recognized as a distinct species by Stossich (1896).

The genus *Toxascaris* was established by Leiper (1907) to contain the arrow-headed ascaridoids with smooth eggs and without an oesophageal ventriculus; *Ascaris leonina* von Linstow 1902 was designated the type species. Leiper included *A. tigridis* Gmelin and *A. marginata* Rudolphi in the genus, but this was erroneous; first, because there is no adequate description of *A. tigridis*, and secondly because, as Railliet & Henry (1911) pointed out, *A. marginata* Rudolphi did not have the characters of *Toxascaris*. Railliet & Henry (1911) accepted von Linstow's species, *A. leonina*, as the type species of *Toxascaris*, and included two other species in the genus, namely, *T. limbata* from the dog and *T. microptera* from the wolf. The former was a new species, while the latter was Rudolphi's species (Rudolphi, 1819) which they had re-examined from his original specimens in the Vienna Museum. Leiper (correspondence) has also re-examined *Ascaris microptera* Rudolphi and has stated that it has smooth-shelled eggs. As *A. microptera* has been shown to belong in the genus *Toxascaris*, and it is concluded that the species of *Toxascaris* occurring in the wolf is identical with *T. leonina*, it follows by the law of priority that *T. leonina* is a synonym of *T. microptera*.*

Throughout the nineteenth century, helminthologists had reported, under various names, the occurrence of winged ascaridoids in a wide variety of Canidae and Felidae. They described only the external features, so that it is not possible in many instances to identify what they actually found. This applies particularly to the ascaridoids of Canidae, because, as a general rule, there are two distinct groups of winged species in these animals, i.e. those belonging to the genus *Toxascaris*, having no oesophageal ventriculus and smooth egg-shells, and those belonging to the genus *Toxocara*, having an oesophageal ventriculus and pitted egg-shells. The species of *Toxocara* which usually occurs in Canidae, namely, *Toxocara canis*, has narrow cervical alae and superficially resembles *Toxascaris* spp. very closely. Hence it is not possible to ascertain, from a description which contains no reference to the oesophagus or egg, to which of these two genera the species described by the early helminthologists should be designated.

The position is somewhat different in regard to the ascaridoids of Felidae, because *Toxocara cati* has broad, short alae which are superficially quite distinct from *Toxascaris* spp. Hence it usually follows that an ascaridoid recorded from Felidae and described as possessing narrow cervical alae, belongs to *Toxascaris*.† With these considerations in mind, the synonymy of *Toxocara cati* has been listed (Sprent, 1956), but the synonymy of *T. canis* cannot be compiled from descriptions prior to Leiper (1907), unless differentiation from *Toxascaris leonina* can be established. For the same reason, there is doubt of the identity of the original specimen

* As the name *Toxascaris leonina* has been in general use for more than 50 years, it appears that the appropriate course would be the application of the Principle of Conservation of the New Code of Zoological Nomenclature (Article 5). Accordingly it is intended to make application to the International Commission on Zoological Nomenclature for the retention of *T. leonina* and the suppression of *Ascaris leptoptera* and *A. microptera*.

† *T. canis* is very rarely reported in *Felidae*.

of the type species of *Toxocara*, i.e. *Lumbricus canis* Werner, 1782; and, unless the original material is available for redescription, the following species must be regarded as nomina dubia: *Ascaris caniculae* Schrank, 1788; *A. canis* Gmelin, 1790; *A. werneri* Rudolphi, 1793; *A. mystax canis* Railliet, 1893; *A. vulpis* Frölich, 1789; *A. triquetra* Schrank, 1790; *A. canis-lagopodis* Viborg, 1795; *A. canis-aurei* Rudolphi, 1819; *A. lupi* Gmelin, 1790.

In compiling a list of synonymy for *Toxascaris leonina*, the same considerations have been kept in view. The following list was compiled as a result of perusal of the original descriptions or redescriptions. In some instances there was conflicting evidence of identity; inclusion or not in the list of synonymy was based on the impression received from the overall description as to whether or not the worm in question was *T. leonina*. It has been concluded from the findings of Taylor (1924) and from those recorded in this paper that, although there is some evidence to

Table 1. *Host list for* Toxascaris leonina*

| | | |
|---|---|---|
| *Alopex lagopus* | Arctic fox | Vevers, 1923 |
| *A. beringensis* | — | Mozgovoi, 1953 |
| *Canis aureus* | Jackal | Railliet & Henry, 1911 |
| *C. dingo* | Dingo | Canavan, 1929 |
| *C. familiaris* | Dog | Railliet & Henry, 1911 |
| *C. latrans* | Coyote | Canavan, 1931 |
| *C. lupus* | Wolf | Furmaga & Wysocki, 1949 |
| *C. occidentalis* | Arctic wolf | Vevers, 1923 |
| *Lycaon pictus* | Cape hunting dog | Canavan, 1931 |
| *Nyctereutes procyonoides* | Raccoon dog | Mozgovoi, 1953 |
| *Urocyon cinereoargenteus* | Grey fox | Caballero, 1937 |
| *Vulpes vulpes* | European fox | Kreis, 1952 |
| *V. fulva* | American red fox | Smith, 1943 |
| *V. bengalensis* | Indian fox | Baylis & Daubney, 1922 |
| *V. macrotis* | — | Hannum, 1943 |
| *V. regalis* | American prairie red fox | Smith, 1943 |
| [*Acinonyx*] *jubatus* | Cheetah | Baylis & Daubney, 1922 |
| [*Lynx*] *canadensis* | Canada lynx | McClure, 1934 |
| [*L.*] *rufus* | American wildcat | Canavan, 1931 |
| [*L.*] *rufus texensis* | American spotted wildcat | Canavan, 1929 |
| *?Felis bengalensis* | Leopard cat | Baylis & Daubney, 1922 |
| *F. catus* [*silvestris ?*] | European wildcat | Canavan, 1931 |
| *F. concolor* | Puma | Canavan, 1931 |
| *F. catus domestica* | Domestic cat | Taylor, 1924 |
| *F. macroura* | South American tiger cat | Stossich, 1896 |
| *F. mitis* | Brazilian chati | Stossich, 1896 |
| *F. ocreata cafra* | Kaffir cat | Le Roux, 1934 |
| *F. pardalis* | Ocelot | Carvalho, 1940 |
| *F. serval* | African serval | Railliet & Henry, 1911 |
| *F. viverrina* | Fishing cat | Baylis & Daubney, 1922 |
| [*Panthera*] *uncia* | Snow leopard | Baylis & Daubney, 1922 |
| [*P.*] *onca* | Jaguar | Diesing, 1851 |
| [*P.*] *tigris* | Tiger | Baylis & Daubney, 1922 |
| [*P.*] *leo* (type host) | Lion | von Linstow, 1902 |
| [*P.*] *pardus* | Leopard | Baylis & Daubney, 1922 |

* Also recorded from man by Leiper (1907).
? indicates a doubtful record.
[ ] indicates that a different name was used by the author.

indicate the existence of canine and feline strains differing in their early development, *T. leonina* can be regarded as a uniform species occurring in the wolf, dog and lion. Hence *T. limbata* and *T. microptera* are regarded as synonyms. A list of hosts for the adult stage is given in Table 1.

*Toxascaris leonina* (von Linstow, 1902), Leiper 1907

*Ascaris teres* Goeze, 1782, p. 80 (Keine Backenbärtige).

*A. crenata* Schrank, 1790, p. 121; not *A. crenata* Rudolphi, 1809, pp. 146–7.

*A. cati* Gmelin, 1790, p. 3031; not *A. cati* Schrank, 1788, p. 8.

*A. leptoptera* Rudolphi, 1809, p. 137, pl. I, figs. 12–13; not *A. leptoptera* Rudolphi, 1819, p. 274; not *A. leptoptera* (Rudolphi) of Schneider, 1866, pp. 39–40, pl. I, fig. 5.

*A. microptera* Rudolphi, 1819, p. 275 (re-examined by Railliet & Henry, 1911, p. 15).

*A. marginata microptera* Dujardin, 1845, p. 160.

*A. felis-discoloris* Leidy, 1851, p. 155.

*A. anterospiralis* Molin, 1860, p. 337 (re-examined by von Drasche, 1883, pp. 130–1, pl. X, figs. 14, 15).

*A. leonina* von Linstow, 1902, pp. 217–18, pl. XIII, fig. 1.

*Toxascaris marginata* (Rudolphi) of Leiper, 1907, p. 1297.

*Ascaris leptoptera* (Rudolphi) of Smith, Fox & White, 1908, pp. 285–7, pl. IV, figs. 1–8.

*A. canis* (Werner) Glaue, 1909, pp. 785–90, figs. 1(*b*), 2(*b*), 3(*b*).

*Toxascaris leonina* (von Linstow) Railliet & Henry, 1911, p. 15.

*T. limbata* Railliet & Henry, 1911, p. 15.

*T. microptera* (Rudolphi), Railliet & Henry, 1911, p. 15.

Chatin, 1879, pp. 266–7 described some worms from a lion, but did not name them, it appears that they should be included in the above list.

### (*b*) *Life history*

Nelson (1852) made a detailed study of oogenesis, fertilization and development of the egg of '*Ascaris mystax*', but it appears from his figures that *Toxascaris leonina* was the subject of his investigation. Fülleborn (1922) fed eggs of *T. leonina* to a dog and 13 days later found partly encysted larvae in the liver, lungs and alimentary tract. These larvae measured 0·210–0·700$\mu$; some were moulting. Larvae in the liver of this dog were fed to a dog which was killed 8–9 days later. Larvae were found in the intestine, but there was no evidence of growth. Fülleborn observed that many larvae reached the abdominal cavity and mesenteric lymph nodes, while relatively few reached the liver and lungs; he concluded that the larvae reached the abdominal tissues by penetration through the intestinal wall.

Wright (1935) studied the development and migration of *T. leonina* in experimental animals and found that the eggs hatched in the duodenum, completed the first moult, and penetrated the small intestine. While remaining in its wall, the larvae underwent considerable growth; and emerged again at 9–10 days after infection. Wright stated that the second moult probably occurs on emergence into

the intestinal lumen and the third moult at 18 days after infection. He observed that some larvae reached the mesenteric lymph nodes and other locations and that they reached the third stage, but he concluded that the larvae do not regularly migrate throughout the body.

With regard to the migration of the larvae of *T. leonina* in rodents, Fülleborn observed that the larvae reached the mesenteric tissues of guinea-pigs. Schulz (1931) found immature *T. leonina* in the liver of a rabbit, presumably as a result of natural infection. Sprent (1952) showed that the larvae of *T. leonina* underwent a preliminary migration into the wall of the intestine, but that after about a week migrated to the somatic tissues. Sprent (1953*b*) reported infection of cats, through feeding mice harbouring the larvae in the tissues.

This paper records observations which have been made on larval forms of *T. leonina*, obtained from dogs and cats following experimental infections, and upon adult specimens of *T. leonina* which have been obtained from various wild and domestic carnivores. The purpose of the work was to compare the development and life history of *T. leonina* with that of *Toxocara canis* and *T. cati* (see Sprent, 1956, 1958) and to establish by morphological studies and by cross-infection experiments whether a single species infects both Felidae and Canidae.

## II. METHODS

Adult specimens have been collected by the writer from various animals in Canada and Australia. The writer is indebted to Dr Robert Rausch, United States Public Health Service, for sending a fine collection of preserved specimens from various hosts in Alaska. The feline strain of *Toxascaris leonina* was originally obtained from the lynx (*Lynx canadensis*) and was maintained for several years in cats. The canine strain of *Toxascaris leonina* was obtained from a naturally infected dog in Brisbane; no natural feline infections have been observed in Australia. Wolf and fox strains of *T. leonina* were obtained from natural infections in Ontario, Canada.

Eggs were dissected from the uteri of female worms and were kept in moist charcoal at room temperature. Experimental infection was produced in mice by mixing the eggs with the food in small dishes as previously described (Sprent, 1952). All dogs and cats were given anthelmintic treatment before experimental infection; this treatment consisted of 50–100 mg. diethylcarbamazine ('Banocide'). Dogs and cats were infected by mixing cultured embryonated eggs with meat, also by feeding minced infected mouse tissues. In four infections (C25, C26, C27 and C28), eggs in cat faeces were used to produce infection; these faeces also contained eggs of *Toxocara cati*.

Experimental animals were killed with ether and the various tissues were digested as previously described (Sprent, 1956). The contents of the alimentary tract were washed out with physiological saline and the worms collected by sedimentation. Larvae were fixed by adding an equal quantity of boiling 6% formalin to the fluid containing them. Adults were fixed in hot 70% alcohol. Larvae were transferred to a mixture of 5% glycerin in 70% alcohol. This fluid was evaporated to the point where the larvae could be transferred in a drop to a slide and a coverslip was applied. As further evaporation took place, pure

glycerin was run in beneath the coverslip, which was then 'ringed' with finger-nail varnish. Adult worms were cleared with creosote for examination. Tissues were fixed in Zenker-Helly fixative or in 10% formalin and stained with haematoxylin and eosin or haematoxylin-eosin-azure II.

## III. EXPERIMENTAL INFECTIONS

### A. Mice

It has already been demonstrated (Sprent, 1952) that the larvae of *Toxascaris leonina* migrate to the wall of the intestine of mice following ingestion of the eggs, and that after about a week they leave the intestinal wall and appear in the carcass. In the previous experiments, eggs from a feline strain were used. This experiment was repeated both with a feline and canine strain of *T. leonina*, and the previous results were confirmed in each instance, indicating that the migratory behaviour of both strains is similar. Tables 2 and 3 show the numbers of larvae recovered

Table 2. *Larvae recovered from tissues of mice infected with* 5000 Toxascaris leonina *feline-strain eggs*

| Mouse no. | Days | Carc. | Liver | Lung | Int. wall | Int. contents | Length range (mm.) |
|---|---|---|---|---|---|---|---|
| 2095 | 1 | 0 | 2 | 1 | 241 | 5 | 0·185–0·220 |
| 2083 | 2 | 0 | 0 | 8 | 165 | 20 | 0·280–0·331 |
| 2084 | 3 | 3 | 2 | 0 | 320 | 1 | 0·252–0·325 |
| 2085 | 4 | 0 | 3 | 0 | 204 | 4 | 0·311–0·446 |
| 2086 | 5 | 3 | 8 | 0 | 410 | 4 | 0·365–0·491 |
| 2087 | 6 | 1 | 12 | 3 | 180 | 0 | 0·410–0·572 |
| 2096 | 7 | 156 | 67 | 48 | 489 | 12 | 0·455–0·567 |
| 2097 | 8 | 117 | 121 | 111 | 51 | 1 | 0·495–0·544 |
| 2088 | 9 | 250 | 20 | 6 | 6 | 0 | 0·425–0·609 |
| 2089 | 10 | 410 | 37 | 31 | 1 | 0 | 0·468–0·585 |
| 2090 | 11 | 456 | 12 | 11 | 1 | 0 | 0·441–0·585 |
| 2092 | 12 | 500 | 20 | 52 | 3 | 0 | 0·495–0·608 |
| 2093 | 13 | 1216 | 20 | 12 | 6 | 0 | 0·473–0·639 |
| 2099 | 14 | 580 | 23 | 13 | 3 | 0 | 0·481–0·571 |

from the various tissues of two series of fourteen mice, which had been given 5000 eggs from a feline and canine strain of *T. leonina*, respectively. The mice were killed on consecutive days and the larvae were collected and counted. For the first 7 days after infection, the length of ten larvae from the intestinal wall of each mouse was measured, for the second week the length of ten larvae from the carcass was measured. The range of length measurements is shown in Tables 2 and 3.

A small number of eggs from a wolf strain and a fox strain of *T. leonina* made it possible to compare the larvae of these strains with the feline and canine strains. Eggs were fed to five mice which were killed at 1, 3, 5, 7 and 10 days, respectively, in the case of the wolf strain, and at 4, 6, 8 and 12 days in the case of the fox strain. The range of length measurements is shown in Tables 4 and 5 and indicates a close resemblance to the canine series. The larvae appeared identical with larvae

of the two other strains. In the wolf-strain infected mouse killed at 10 days, several of the larvae had a double cuticular outline.

It was evident that the larvae of *T. leonina* entered the intestinal wall of mice and underwent a period of growth; the canine, wolf and fox strains grew slightly more rapidly and attained a slightly greater length than the feline strain. At the

Table 3. *Larvae recovered from tissues of mice infected with* 5000 Toxascaris leonina *canine-strain eggs*

| Days after infection | Mouse | Carc. | Liver | Lung | Int. wall | Int. contents | Length range (mm.) |
|---|---|---|---|---|---|---|---|
| 1 | 2056 | 0 | 0 | 3 | 77 | 0 | 0·206–0·245 |
| 2 | 2064 | 1 | 0 | 0 | 249 | 13 | 0·351–0·464 |
| 3 | 2065 | 1 | 0 | 0 | 221 | 17 | 0·324–0·419 |
| 4 | 2057 | 0 | 0 | 0 | 192 | 7 | 0·477–0·554 |
| 5 | 2058 | 0 | 0 | 0 | 766 | 21 | 0·608–0·653 |
| 6 | 2059 | 27 | 25 | 8 | 710 | 21 | 0·590–0·648 |
| 7 | 2060 | 550 | 73 | 18 | 64 | 1 | 0·599–0·700 |
| 8 | 2061 | 1200 | 9 | 1 | 3 | 0 | 0·599–0·684 |
| 9 | 2066 | 1175 | 13 | 12 | 4 | 2 | 0·612–0·686 |
| 10 | 2067 | 2100 | 33 | 2 | 0 | 1 | 0·610–0·707 |
| 11 | 2062 | 660 | 4 | 6 | 1 | 1 | 0·553–0·686 |
| 12 | 2063 | 769 | 4 | 0 | 0 | 0 | 0·595–0·671 |
| 13 | 2068 | 2430 | 2 | 1 | 1 | 0 | 0·579–0·681 |
| 14 | 2069 | 1375 | 5 | 2 | 1 | 2 | 0·531–0·661 |

Table 4. *Length measurements (mm.) of larvae from mice infected with a wolf strain of* Toxascaris leonina

| Mouse no. | Days | Length (range) |
|---|---|---|
| 1103 | 1 | 0·231–0·287 |
| 1106 | 3 | 0·361–0·427 |
| 1104 | 5 | 0·371–0·574 |
| 1105 | 7 | 0·480–0·700 |
| 1107 | 10 | 0·542–0·700 |

Table 5. *Length measurements (mm.) of larvae from mice infected with fox strain of* Toxascaris leonina

| Mouse no. | Days | Length (range) |
|---|---|---|
| 1191 | 4 | 0·28–0·35 |
| 1192 | 6 | 0·47–0·74 |
| 1193 | 8 | 0·54–0·72 |
| 1194 | 12 | 0·56–0·80 |

end of a week the larvae began to migrate to the carcass and to undergo the second moult. The feline strain larvae retained the loose cuticle up to 14 days, whereas the canine strain evidently completed the moult at about 9 days and appeared as third-stage larvae. Four mice were infected with feline strain eggs and two were killed at 21 and 28 days after infection, respectively. The larvae recovered from these mice had lost the cuticular sheath and were evidently third-stage larvae; their length was 0·52–0·65 mm. This indicated that the feline-strain

larvae completed the second moult in mouse tissues, but at a later stage of infection.

About 5 days after infection, white spots and streaks appeared on the surface of the intestine. At 6 days the lungs began to have a mottled appearance, and from 7 to 12 days after infection, distinct red spots appeared, and the whole lung was more or less congested in appearance. At about 10 days white spots began to appear on the viscera and subcutaneous tissues. These white spots were the size of a pin-head and each contained a coiled larva. They occurred along the back, on the abdominal wall, costal region, neck and occipital region, as well as on the surface of internal organs such as the liver, heart, intestine, caecum, rectum, diaphragm, and the retroperitoneal and perirectal tissues.

In three series of mice which were infected with eggs of *T. leonina* (see above), portions of the small intestine and in some instances other tissues, were fixed for histological study. The sections confirmed the results obtained by larval counts, i.e. that for the first week after infection the larvae remained in the intestinal wall and thereafter migrated to the somatic tissues.

On the day after infection the larvae had already penetrated the muscularis mucosae and were distributed throughout the submucosa, especially in the circular muscle zone (Pl. VII, fig. 10). Each larva was associated with a dense cluster of heterophil leucocytes and lymphoid cells; eosinophil leucocytes were scarce. Many of these granulomatous clusters were elongated at right angles to the surface, indicating that the larvae had moved across the submucosa. No larvae were observed in the epithelial layer, which appeared to be normal.

At 2 days there was an increased preponderance of heterophil leucocytes and an accumulation of pink-staining debris within the clusters (Pl. VII, fig. 11). In the submucosa, in the vicinity of the clusters, there was an infiltration of cells of the lymphoid-macrophage series and eosinophil and heterophil leucocytes. At 3 days some of the cell clusters had begun to show differentiation into a central zone of leucocytes and a peripheral zone of lymphoid-macrophage elements. At 5 days several larvae, unassociated with any cellular infiltration, indicated that they commenced migration at this time. This was further indicated by the observation that after 6 days many of the cell clusters contained no larvae, but consisted mostly of macrophages and fibroblasts; around such nodules there were fibrous strands (Pl. VII, fig. 12). As the duration of infection increased, the granulomatous clusters in the intestinal wall became less conspicuous, because the larvae had evidently migrated away from the intestine; consequently, leucocytic infiltration diminished and the lesions were reduced to scar tissue. Sections of the liver were studied over the same period. The liver appeared normal throughout, except for a few granulomatous clusters, streaks of leucocytic infiltration, and periportal infiltrations at 10–12 days; the cells involved were heterophil and eosinophil leucocytes. The lungs appeared normal up to 8 days when areas of alveolar haemorrhage began to appear, at 10 days there was peribronchial infiltration mostly with eosinophil leucocytes.

At 13 days after infection, larvae were observed in a section of abdominal wall. There was massive infiltration of the surrounding tissues with eosinophil leucocytes

and the larvae were embedded within dense clusters of heterophil and eosinophil leucocytes and lymphoid-macrophage cells, the former being mostly aggregated in the centre (Pl. VII, fig. 13).

A larva was observed in sections of heart taken at 28 days. The larva was embedded in a dense granuloma with a mass of eosinophils in the centre, the lesion was elongated parallel with the muscle fibres.

## B. CATS

Cats were infected by two methods: (1) by feeding with embryonated eggs, (2) by feeding tissues of mice harbouring larvae in their tissues. Using four groups of cats, eggs of feline and canine strains of *T. leonina* were used for each method of infection.

### (1) *Infection by feeding eggs to cats*

#### (*a*) *Feline strain*

Fourteen cats were infected by feeding eggs of the feline strain mixed with meat. These cats were killed at various intervals after infection, and, unless stated to the contrary, the liver, lungs, muscle, stomach wall, stomach contents, intestinal wall and intestinal contents were searched for larvae. In four infections (C25, C26, C27 and C28) the egg cultures were contaminated with eggs of *Toxocara cati*.

5 *days*. An 8-week-old cat (C16) was given 2000 eggs. 5 days later it died. One second-stage larva (length 0·28 mm.) was found in the intestinal wall.

7 *days*. An 8-week-old cat (C25) was given 2000 eggs and killed 7 days later. Many second-stage larvae (length 0·33–0·50 mm.) were found in the intestinal wall and some of these were moulting (length 0·42–0·47 mm.); many second-stage larvae (length 0·37–0·50 mm.) were found in the intestinal contents (*T. cati* larvae were also found).

11 *days*. A young cat (C78) was given 1000 eggs; 11 days later it died. Liver and lungs were not examined. One second-stage larva was found in the stomach wall and fourteen second-stage larvae (length 0·36–0·49 mm.) were found in the intestinal wall; in all these larvae the cuticle had a double outline, one larva was moulting (length 0·45 mm.).

14 *days*. An 8-week-old kitten (C26) was given 2000 eggs and killed 14 days later. Many (not counted) larvae (length 0·58–0·64 mm.) were found in the intestinal wall; in all of them the cuticle had a double outline, resembling feline-strain larvae observed in mouse tissues and they were regarded as second-stage larvae. Similar larvae (length 0·51–0·60 mm.), but with anterior projections, were found in the intestinal contents; the cuticle was loose in many of these larvae. There were also four fourth-stage larvae (length 0·57–0·77 mm.) which indicated that some of the moulting larvae in the intestinal contents may have been third moults, but this could not be ascertained. *T. cati* larvae were also found.

14 *days*. A young cat (C86) was given 5000 eggs and was killed 14 days later. There were nineteen second-stage larvae (length 0·43–0·56 mm.) in the intestinal wall resembling feline-strain larvae in mouse tissues at 14 days; some of the larvae had a double cuticle and some had an anterior projection (length 0·55–0·56 mm.).

21 *days*. An 8-week-old kitten (C27) was given 2000 eggs and killed 21 days later. In the intestinal wall there were eight third-stage larvae (length 0·58–0·73 mm.), some moulting (length 0·68–0·73 mm.), and also three fourth-stage larvae (length 0·64–0·67 mm.). In the intestinal contents there were ten third-stage larvae (length 0·63–0·68 mm.) and five fourth-stage larvae (length 0·67–0·75 mm.) (*T. cati* larvae were also found).

21 *days*. A young cat (C87) was given 5000 eggs and killed 21 days later. One damaged larva (length 0·62 mm.) was found in the lungs. There were twenty-five larvae in the intestinal wall: they were all slightly damaged, but appeared to be fourth-stage larvae, their length being 0·61–0·68 mm. In the intestinal contents were twenty-three larvae in various stages of growth, i.e. moulting third-stage larvae (length 0·68–0·71 mm.), and fourth-stage larvae (length 0·65–2·9 mm.).

28 *days*. A young cat (C88) was given 5000 eggs and killed 28 days later. There were 193 larvae in the intestinal wall: all were fourth-stage larvae (length 0·60–0·72 mm.), but many of them had loose cuticles. There was one fourth-stage larva (length 1·6 mm.) in the stomach contents. There were 177 larvae in the intestinal contents; these were in various stages of growth, i.e. third-stage larvae (0·66–0·69 mm.), small fourth-stage larvae (length 0·67–0·74 mm.), larger fourth-stage larvae (length 1·3–6·6 mm.), and adults (length 8·4–10·9 mm.).

35 *days*. A young cat (C90) was given 5000 eggs and killed 35 days later. One larva (lost) was found in the muscle. Seventy fourth-stage larvae (length 0·60–0·90 mm.) were found in the intestinal wall, several still in the third moult. In the intestinal contents there were fifty fourth-stage larvae in various stages of growth (length 0·69–5·5 mm.).

42 *days*. A young cat (C91) was given 5000 eggs and killed 42 days later. In the stomach contents there was a fourth-stage larva (length 8·6 mm.) and a moulting fourth-stage larva (length 7·7 mm.). In the intestinal wall there were seventy-nine fourth-stage larvae (length 0·43–0·82 mm.); many of them had not completed the third moult. In the intestinal contents there were eight small fourth-stage larvae (length 0·71–0·72 mm.), two larger fourth-stage larvae (length 4·2 and 6·0 mm.), and two adults (length 16·6 and 16·7 mm.).

42 *days*. An 8-week-old kitten (C28) was given 1000 eggs and killed 42 days later. There were many fourth-stage larvae (length 0·65–0·71 mm.) in the intestinal wall. In the intestinal contents were many fourth-stage larvae (length 0·63–2·7 mm.). A number of adult *T. cati* were also found.

In three cats the infection was allowed to develop to the stage of egg-laying: An 8-week-old kitten (C17) was given 1000 eggs. The faeces were not examined until 4 months later when *Toxascaris leonina* eggs were found. At 6 months after infection, adult *T. leonina* were passed. This cat was kept as a source of eggs for several months.

A young cat (C65) was given 1000 eggs of *T. leonina* and the faeces were examined weekly for the appearance of eggs. Eggs of *T. leonina* appeared 74 days after infection. 7 months after infection, several adult *T. leonina* were passed in the faeces and the cat died 6 days later. In the stomach and intestinal contents were sixty-four larvae and four adult *T. leonina*; these were mislaid and not measured.

An 8-week-old kitten (C35) was given 3000 eggs of *T. leonina* and 3000 eggs of *Toxocara cati*. This was repeated four times at weekly intervals. Thereafter the faeces were examined at weekly intervals. No eggs were observed for 4 weeks after the last infection, at which time eggs of *T. cati* appeared and continued to appear for 10 weeks. For a further 8 weeks no eggs were found. At 22 weeks after infection *Toxascaris leonina* eggs began to appear and continued to appear for 38 weeks after infection. The cat was killed a year after infection and in the intestinal contents there were sixteen small fourth-stage larvae (length 0·52–0·74 mm.), five larger fourth-stage larvae (length 3·7–5·9 mm.), and three adults (19–39 mm.); all were larvae of *T. leonina*. The reason for giving a mixed infection to this cat was to find whether the presence of *Toxocara cati* inhibited the development of *Toxascaris leonina*. The findings, compared with the previous infection (C65), indicated that egg-laying was delayed. An inhibitory influence of *Toxocara cati* is also indicated by a comparison of the stage of development of *Toxascaris leonina* in C91 and C28, which were both killed at 42 days after infection, and in C21 and C87 which were both killed at 21 days after infection (see above).

(*b*) *Canine strain*

Three young cats were given 5000 eggs from a dog strain of *T. leonina*. They were killed at 21, 35 and 49 days after infection, respectively. Liver, lungs, muscle, stomach and intestinal wall and the contents of the stomach and intestine were searched for larvae, but no larvae were found.

(2) *Infection by feeding mice to cats*

(*a*) *Feline strain*

Fourteen cats were infected by feeding them with the tissues of mice which had previously been infected with eggs of a feline strain of *T. leonina*. The cats were killed at various intervals after infection.

7 *days*. A young cat (C79) was infected with the tissues of a mouse (infected 5 months previously with eggs). It was killed 7 days after infection. One damaged larva (length 0·56 mm.) was found in the intestinal wall.

13 *days*. A young cat (C66) was infected with the tissues of a mouse (infected 2 months previously with eggs). It was killed 13 days later. Four third-stage larvae (length 0·54–0·57 mm.) were found in the intestinal contents.

18 *days*. A kitten (C12) was infected with mouse tissues (infected for 28 days); it died 18 days after infection. Three third-stage larvae (length 0·65–0·68 mm.) were found in the intestinal wall. In the intestinal contents there were four third-stage larvae (length 0·61–0·67 mm.).

18 *days*. A kitten (C13) was given mouse tissues (infected for 28 days); it died 18 days after infection. There were nine third-stage larvae (length 0·56–0·70 mm.) and one fourth-stage larva (length 1·3 mm.) in the intestinal contents.

20 *days*. A kitten (C11) was given mouse tissues (infected for 28 days); it died 20 days after infection. There were eight third-stage larvae (length 0·63–0·68 mm.) in the intestinal wall. In the intestinal contents there were two third-stage larvae (length 0·64 and 0·685 mm.) and two fourth-stage larvae (length 0·71 and 0·74 mm.).

21 *days.* A young cat (C67) was given mouse tissues (infected for 2 months); it was killed 21 days later. One third-stage larva (length 0·59 mm.) was found in the stomach contents, and one third-stage larva (length 0·60 mm.) in the intestinal contents.

21 *days.* A young cat (C81) was given mouse tissues (infected for 30 days); it was killed 21 days later. Fifteen second-stage larvae (length 0·54–0·62 mm.) were found in the intestinal wall; these still retained the cuticle and appeared identical with larvae found in mouse tissues. Two third-stage larvae (length of one 0·64 mm.) were found in the stomach contents. In the intestinal contents there were twelve larvae in various stages of growth, i.e. a second-stage larva (length 0·47 mm.), third-stage larvae (length 0·61–0·62 mm.) and fourth-stage larvae (length 1·4–4·9 mm.).

28 *days.* A kitten (C18) was given mouse tissues (infected for 2 months); it was killed 28 days later. Two third-stage larvae (length 0·63 and 0·68 mm.) and one fourth-stage larva (length 0·74 mm.) were found in the intestinal contents.

35 *days.* A young cat (C82) was given mouse tissues (infected for 30 days); it was killed 35 days later. There were nine larvae in the intestinal wall (two moulting third-stage larvae measured 0·63 and 0·65 mm.; three fourth-stage larvae measured 0·64–0·70 mm.) There were fourteen larvae in the intestinal contents, some were in the third stage (length 0·63–0·72 mm.) and some in the fourth stage (length 0·65–0·70 mm.).

49 *days.* A young cat (C83) was given mouse tissues (infected for 30 days); it was killed 49 days later. There were two larvae (damaged) in the intestinal wall and four fourth-stage larvae (length 0·65–0·73 mm.) in the intestinal contents.

(*b*) *Canine strain*

Two cats were infected by feeding them with tissues of mice which had previously been infected with eggs of a canine strain of *T. leonina*. These cats were killed at 35 and 49 days after infection, respectively.

35 *days.* A young cat (C84) was given mouse tissues (infected for 5 weeks); it was killed 35 days later. Four third-stage larvae were found in the muscle (length of one—0·75 mm.). In the intestinal wall there were sixty-three fourth-stage larvae (length 0·77–0·84 mm.); many of these larvae had loose cuticles. In the intestinal contents there were forty-nine small fourth-stage larvae (length 0·87–0·98 mm.), five larger fourth-stage larvae (length 2·5–3·2 mm.) and one moulting fourth-stage larva (length 8·8 mm.).

49 *days.* A young cat (C85) was given mouse tissues (infected for 5 weeks); it was killed 49 days later. Four third-stage larvae were found in the muscle (length 0·77–0·79 mm.). In the intestinal wall there were thirty-six fourth-stage larvae (length 0·74–0·88 mm.). In the stomach contents there were three fourth-stage larvae (length 0·86–1·0 mm.), and in the intestinal contents there were seventy-nine fourth-stage larvae (length 0·75–1·1 mm.).

In summarizing the results of these experiments on cats, it was evident that, in the case of egg-infected cats, the larvae remained in the second stage for 2 weeks after infection; they were found in the wall and contents of the intestine, but

rarely in the stomach. The second moult was observed at 11 days in the wall of the intestine. Third-stage larvae appeared 2 weeks after infection. The third moult was observed in the intestinal wall at 2 weeks after infection and fourth-stage larvae were present at this time. Small adults had appeared at 4 weeks after infection, and the fourth moult was observed in the intestinal contents at 6 weeks. Eggs were observed in the faeces at 74 days after infection and continued to appear for 38 weeks in one cat.

A characteristic feature of these infections was the persistence of the small fourth-stage larvae in the wall and contents of the intestine. They were still present at 6 weeks after infection (C28, C91) and in the cat (C35) which was killed a year after infection, fourth-stage larvae were still present. In the latter instance, it is possible that re-infection could have occurred from faeces adhering to the bars of the cage, in spite of their being regularly cleaned. The persistence of small fourth-stage larvae and the small proportion of adults indicated that some inhibitory influence must be exerted on the growth of the fourth-stage larvae.

The failure to infect cats with eggs from the canine strain of *T. leonina* is difficult to explain. The same batch of eggs was infective to mice, and larvae in these mice proved infective for cats (C84) and (C85).

In mouse-infected cats, the infections appeared to be less successful, in that fewer larvae were recovered. The number of larvae recoverd from cats infected with canine-strain infected mice was greater than from cats infected with feline-strain infected mice. In mouse-infected cats the persistence of fourth-stage larvae was observed up to 7 weeks (C85 and C83). The proportion of larvae which developed to the adult stage was even smaller than in the egg-infected cats.

### C. Dogs

Dogs were infected by feeding eggs of the feline strain and the canine strain, also by feeding tissues of mice infected with the canine strain.

#### (1) *Infection by feeding eggs to dogs*

(*a*) *Feline strain*

Three 5-week-old dogs were infected with 5000 eggs from a feline strain of *T. leonina*. They were killed at 14, 38 and 42 days after infection.

14 *days* (D53). No larvae were found in any tissues.

38 *days* (D54). In the intestinal contents there were eight fourth-stage larvae (length 2·9–9·6 mm.) and one adult (length 10 mm.).

42 *days* (D55). In the intestinal contents there were two fourth-stage larvae (length 6·8 and 8·6 mm.), two moulting fourth-stage larvae (length 9·2 and 12 mm.), and ten adults (length 11–24 mm.).

(*b*) *Canine strain*

Three 5-week-old dogs were infected with 5000 eggs from a dog strain of *T. leonina*. They were killed at 21, 32 and 49 days after infection, respectively.

21 *days* (D56). There were fifty larvae in the intestinal contents, they were all third-stage larvae (length 0·49–0·70 mm.).

32 *days* (D57). There were nineteen larvae in the intestinal wall, some were in the third stage (length 0·60–0·76 mm.) and some were in the fourth stage (length 0·70–0·73 mm.); there was one moulting third-stage larva (length 0·69 mm.). There were 288 larvae in the intestinal contents; these were third-stage larvae (length 0·67–0·82 mm.).

49 *days* (D58). There were nine larvae in the intestinal contents. Only four were available for measurement, of these two were small fourth-stage larvae (length 0·78 and 0·80 mm.) and two were larger fourth-stage larvae (length 1·6 and 5·9 mm.).

(2) *Infection by feeding mice to dogs*

One dog was infected by this method. This dog (D52) was infected by feeding the tissues of a mouse (infected 5 months previously with the eggs of a dog strain of *T. leonina*). The faeces were examined weekly for eggs of *T. leonina*. These first appeared 8 weeks after infection and continued to appear for 36 weeks. At 32 weeks several adult *T. leonina* were passed in the faeces.

These experiments showed that, apart from variations in the rate of development of the larvae, which appeared to bear no relation to the strain used, the feline strain of *T. leonina* was observed to grow to the adult stage in both cats (C88, C91) and dogs (D54, D55) and that the canine strain was observed to grow to the adult stage in dogs (D52) and to the fourth stage in cats (C84).

## IV. DEVELOPMENT OF *TOXASCARIS LEONINA*

Experimental infection of dogs and cats with embryonated eggs indicated that there were at least four morphologically distinct larval forms in the development of *T. leonina*; i.e. (1) the larva in the egg, which on hatching was enclosed in a cuticular sheath; (2) the larva with small lips, a cuticular flange and a knobbed tail; (3) the larva with fourth-stage-type lips and pointed tail; (4) the adult. Thus initially it appeared that the development of *T. leonina* differed from the development of other ascaridoid nematodes in that there were three, rather than four, larval stages. On the other hand, experimental infections in mice indicated that only larvae in the second of the above categories occurred in mouse tissues, and that a moult commenced at 8–10 days after infection, indicated by the fact that many of the larvae had a double cuticle. As the larvae recovered from feline-strain infected mice at 21 and 28 days after infection, and in canine-strain infected mice after 9 days, did not have this double cuticle, it was concluded that the second moult was completed in mouse tissues. It was evident that the larvae found in mouse tissues in the later stages of infection, though they resembled those found in the early stages, actually constituted another larval stage. Furthermore, it was observed that when mice harbouring these larvae were fed to dogs and cats, similar larvae appeared in the intestine and, except in one instance, showed no evidence of a double cuticle. Eventually these larvae, though they did not grow in length, underwent a moult, during which the definitive lips characteristic of ascaridoid fourth-stage larvae were formed. Thus larvae with fourth-stage lips were observed with a length no greater than that of the larvae in mouse tissues.

Such larvae were not observed to moult again until they reached a length of about 10 mm., at which time they assumed the adult form.

It was concluded therefore, that there were actually five stages in the development, i.e. (1) the first-stage larva which developed in the egg, the first moult occurring at hatching; (2) the second-stage larva which underwent growth from about 0·2 to 0·4–0·6 mm. both in the intestinal wall of dogs and cats and in the tissues of mice, the second moult occurring at the end of this period of growth; (3) the third-stage larva which resembled the second-stage larva, but did not undergo any growth in length, the third moult marking the end of this resting stage; (4) the fourth-stage larva which grew in length from about 0·6 to about 10 mm., the fourth moult occurring at the end of this period of growth and sexual differentiation; (5) the adult stage. As the third-stage larva resembled the second-stage larva and underwent no growth or apparent development, it could not be easily distinguished in egg-infected dogs and cats. Its identity was indicated, however, by the occurrence of two kinds of moult in egg-infected cats, one where the enclosed larva resembled the second stage and one where the enclosed larva resembled the fourth stage (cf. Text-figs. 12, 13).

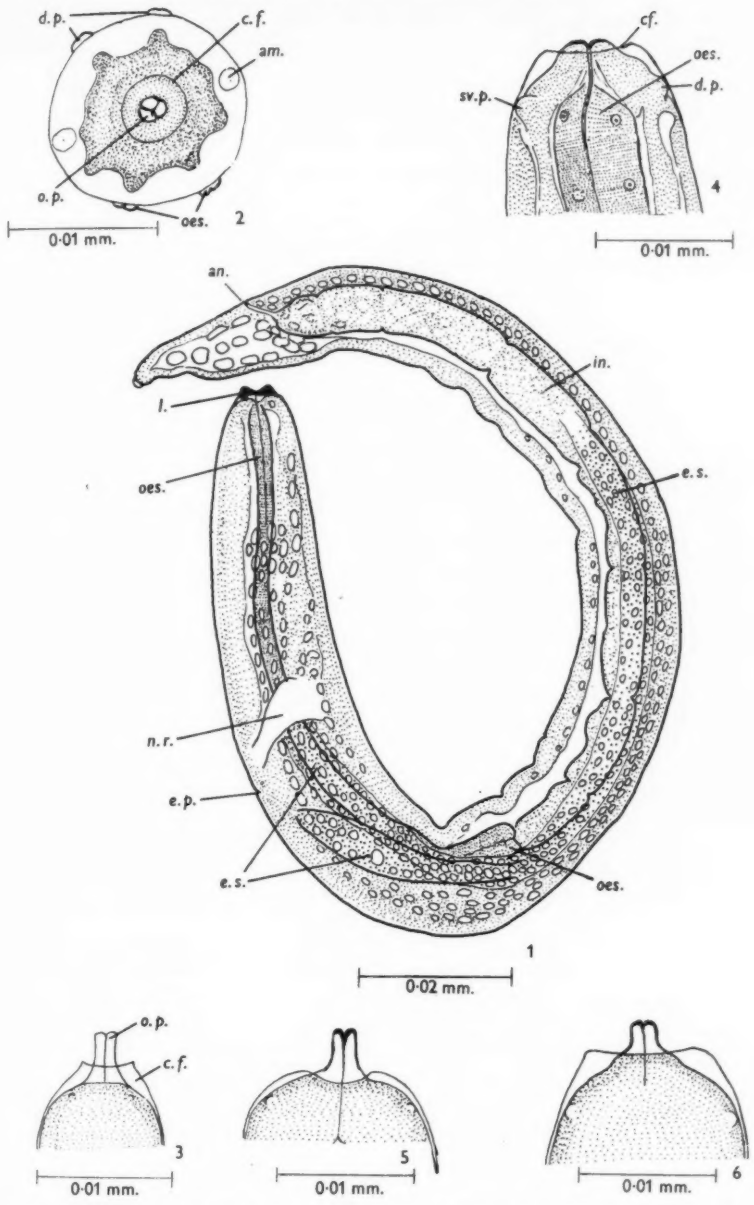
### *The first-stage larva*

The first-stage larvae were observed by releasing them from the eggs by pressure on a cover-slip. The eggs had been removed from the uterus of female worms and had been cultured in moist charcoal for 5 days. The larva was stumpy in appearance, with minute but conspicuous cuticular projections around the mouth. The lateral alae extended from the nerve ring to the tail. The tail terminated in a minute knob. The excretory pore and the anus were already visible; the intestine showed clearly as a row of about eight granular cells, the first two being larger than the others; a few granules were present in the body wall. Five first-stage larvae were measured and the length varied from 0·19 to 0·23 mm.

### *The second-stage larva* (Pl. VI, fig. 1)

After an undetermined period, the cuticle of the first-stage larva becomes loose, so that when the eggs are pressed beneath the cover-slip, the larvae which emerge are enclosed in a cuticular sheath. This larva is the second-stage larva and according to Wright (1935) the cuticular sheath is shed when the larva hatches in the duodenum. Second-stage larvae pressed out of the eggs were stumpy in appearance and on their anterior end had conspicuous cuticularized triangular structures around the mouth which were probably modified lips (Text-fig. 1). The body of the larva is filled with granules which tend to obscure the structural details. The larvae at this stage resemble the first-stage larvae very closely; ten larvae pressed from the egg measured 0·19–0·22 mm. in length. Second-stage larvae were observed in the tissues of mice and in egg-infected cats. They were numerous in the intestinal wall of mice for the first week after infection, and then evidently migrated to the somatic tissues (see Tables 2 and 3).

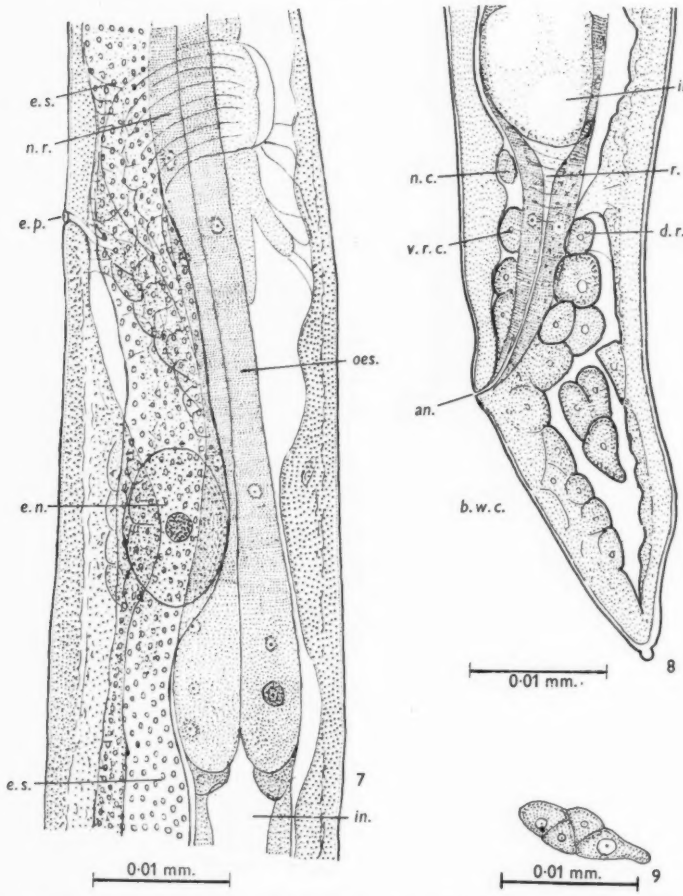
The body of the larva was widest in the anterior third and gradually tapered behind the posterior end of the oesophagus. Behind the anus the tail tapered




*T. leonina*. Text-fig. 1. Second-stage larva pressed from egg (length approx. 0·2 mm.). Text-fig. 2. *En face* view of anterior end of second-stage larva from mouse tissues (length 0·400 mm.). Text-fig. 3. Anterior end of second-stage larva from mouse tissues (length 0·192 mm.). Text-fig. 4. Anterior end of second-stage larva from mouse tissues (length 0·280 mm.). Text-fig. 5. Anterior end of second-stage larva from mouse tissues (length 0·4 mm.). Text-fig. 6. Anterior end of second-stage larva from mouse tissues (length 0·5 mm.).

For list of abbreviations see p. 371.

gradually to end in a minute button-like knob (Text-fig. 8). The anterior end was rounded, with a characteristic prominence formed by the lips, this prominence was elongated to a variable degree (Text-figs. 3–6). This prolongation was frequently observed in second-stage larvae; its presence appeared to bear no relation to the length of the larva or to the tissue in which its was recovered; it being evidently formed by the elongation of the lips, as in *en face* view the tip of




*T. leonina.* Text-fig. 7. Second-stage larva from mouse tissues in region of excretory pore (length 0·280 mm.). Text-fig. 8. Tail region of second-stage larva (length 0·280 mm.). Text-fig. 9. Genital rudiment of second-stage larva (length 0·280 mm.).

the prolongation was clearly divided into three lobes which corresponded with the arrangement of the cephalic papillae. A circular cuticular ridge surrounded the central prominence (Text-fig. 2). This cuticular ridge in side view appeared as two lappet-like projections on either side of the central prominence (Text-fig. 4). The oesophagus commenced at the oral aperture, narrowed at the nerve ring and

gradually widened until it formed a slight bulb at the posterior end, which contained two or more spherical nucleolar particles; there was no evidence of a terminal ventriculus (Text-fig. 7). In the early phase of growth, the intestine consisted of a single row of elongate granular cells (Text-fig. 1). The genital rudiment was an oval body, lying ventrally to the intestine in the posterior third of the body (Text-fig. 9). The excretory pore opened on the ventral surface immediately behind the nerve ring. In mice, growth of second-stage larvae was most evident in the first week of infection (Tables 1 and 2). During this time the internal structure became more distinct. The excretory nucleus became visible on the left side of the posterior third of the oesophagus; the excretory system consisted of two granular bands running almost the whole length of the larvae in the lateral field. At the level of the excretory nucleus, a branch projected forwards towards the excretory pore (Text-fig. 7). As growth proceeded the lumen of the intestine appeared, surrounded by elongate cells, and a number of cells could be defined in the vicinity of the rectum (Text-fig. 8). Length measurements shown in Tables 1 and 2 indicate that the dog-strain larvae attained a greater length in the tissues of mice than feline-strain larvae up to 14 days after infection.

This difference in length was probably associated with the fact that feline-strain larvae collected from mouse tissues from 1 to 14 days after infection appeared to be all second-stage larvae, whereas canine-strain larvae collected after 9 days had undergone the second moult and reached the third stage.

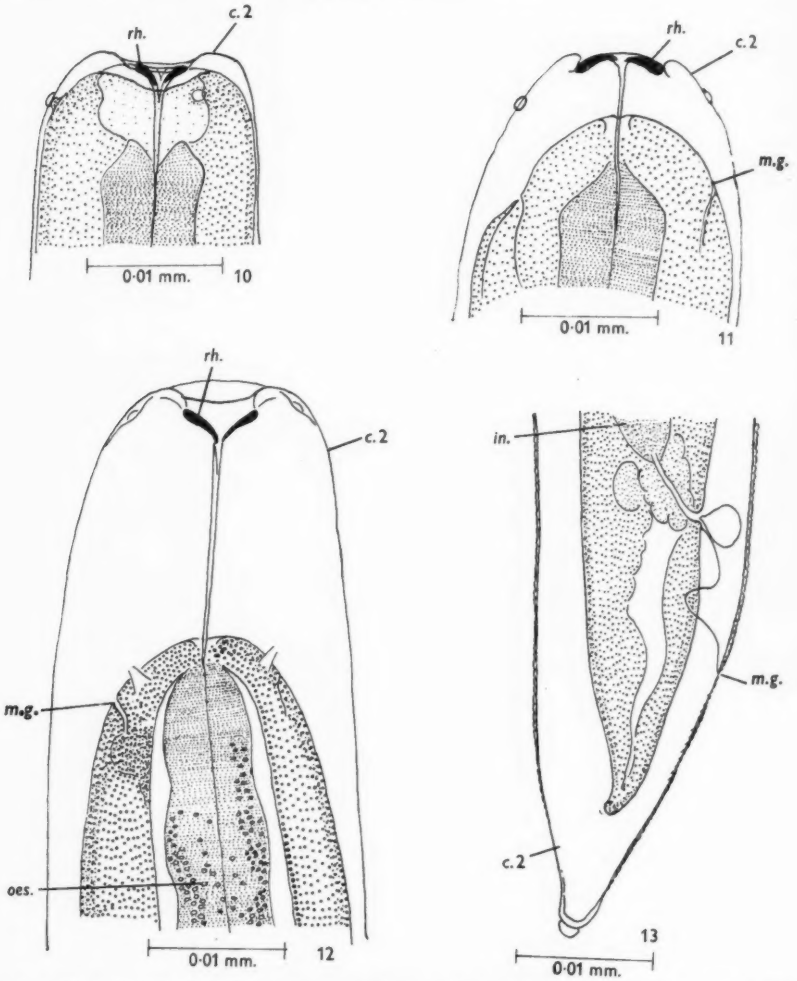
At 8 days in canine-strain infected mice and at 10 days in feline-strain infected mice, several larvae were observed in which the cuticle had a double outline. In the canine-strain series, third-stage larvae were observed in the carcass on the 9th day, whereas in the feline-strain series of mice, larvae with double cuticle were found up to the 14th day. Third-stage larvae were observed at 21 and 28 days in the feline-strain infected mice, indicating that the second moult was completed later in the feline-strain larvae.

Second-stage larvae were observed in the intestinal wall of egg-infected cats up to 14 days after infection; they reached a length of 0·65 mm. In one mouse-infected cat (C81), the larvae in the intestinal wall appeared to be moulting second-stage larvae similar to those found in mouse tissues at 10–14 days after infection. Larvae recovered from other mouse-infected cats were third-stage larvae.

*The second moult* (Text-figs. 10–13)

The second moult was observed in the carcass of feline-strain infected mice at a length of 0·42–0·49 mm. and in the carcass of canine-strain infected mice at 0·51–0·63 mm.; also in the intestinal wall of egg-infected cats at 0·45 (C78), 0·42–0·47 mm. (C25) and 0·55–0·56 mm. (C86). In those larvae where separation had actually occurred at the anterior end, the cuticle of the second-stage larva could be observed with the lining of the mouth and oesophagus still attached to the enclosed larva (Text-figs. 10–12). The rectum of the moulting larva was dilated, its lining was connected with the anus of the separated cuticle and there was sometimes a droplet at the anus; the tip of the tail of the cast sheath was blunt (Text-fig. 13). In some moults the lining of the excretory canal protruded through the

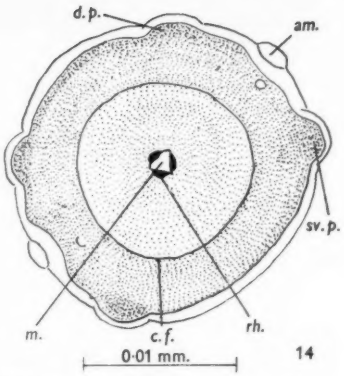
excretory pore of the sheath. The cephalic papillae were continuous with the corresponding structures on the sheath. Another zone of attachment was observed behind the lips of the larva on each side, this appeared to lead into the tissues of the larva; in some instance a distinct break was observed at this point (Text-fig. 11, 12); possibly these structures are glands associated with moulting.

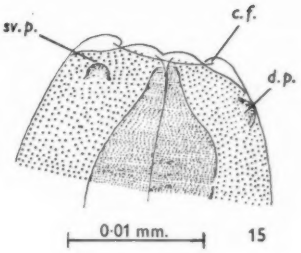


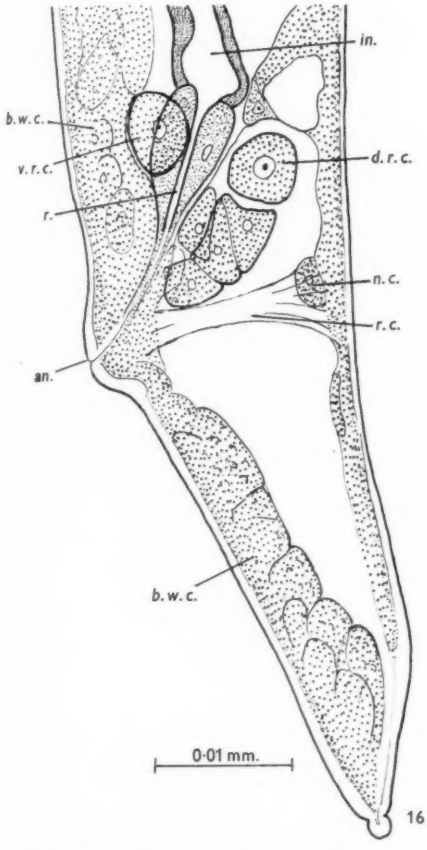

*T. leonina.* Text-fig. 10. Anterior end at second moult (length 0·42 mm.). Text-fig. 11. Anterior end at second moult (length 0·49 mm.). Text-fig. 12. Anterior end at second moult (length 0·45 mm.). Text-fig. 13. Tail region at second moult (length 0·45 mm.).

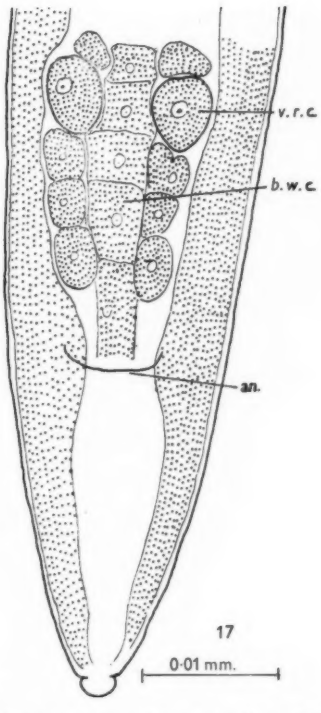
### *The third-stage larva* (Pl. VI, figs. 2, 5)

The third-stage larva closely resembled the second-stage larva, but was more robust in appearance. The cuticular collar was conspicuous, but the circumoral region was more flattened and more thickly covered with cuticle, giving a truncate

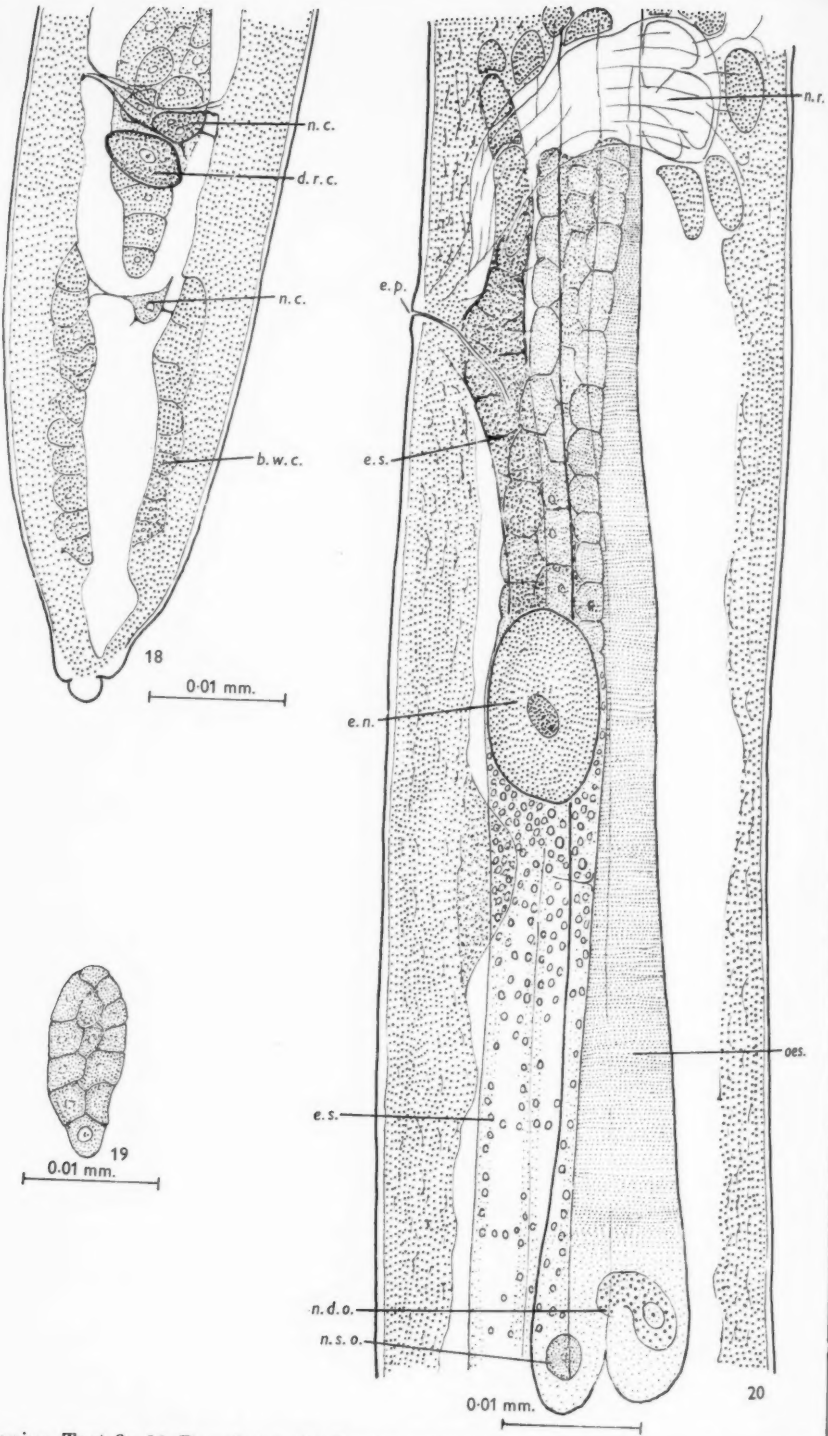



*T. leonina.* Text-fig. 14. *En face* view of anterior end of third-stage larva from intestine of dog (length 0·7 mm.). Text-fig. 15. Anterior end of third-stage larva from tissues of mouse (length 0·62 mm.). Text-fig. 16. Lateral view of tail region of third-stage larva from mouse tissues (length 0·62 mm.). Text-fig. 17. Ventral view of tail region of third-stage larva from mouse tissues (length 0·62 mm.).



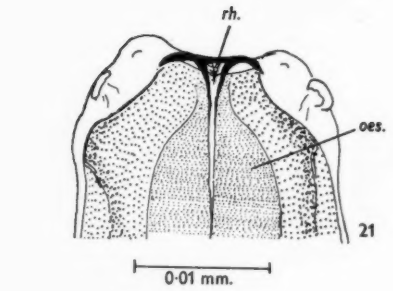

*T. leonina.* Text-fig. 18. Dorsal view of tail region of third-stage larva from mouse tissues (length 0·62 mm.). Text-fig. 19. Genital rudiment of third-stage larva (length 0·62 mm.) from mouse tissues. Text-fig. 20. Region of excretory pore of third-stage larva (length 0·62 mm.).

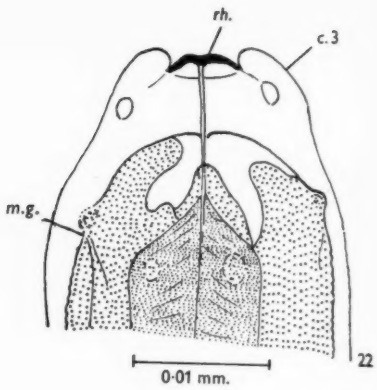
appearance to the anterior end (Text-fig. 15). The cephalic papillae were more prominent (Text-fig. 14). The anterior part of the oesophagus was more granular, the intestinal lumen was relatively wider in the anterior part, the wall being composed of elongate cells. The cells clustered around the rectum (Text-figs. 16–18) could be distinguished as dorsal and ventral groups. The dorsal group was a median row, consisting of an anterior nerve cell, the dorsal rectal cell and a row of smaller cells. The ventral group consisted of a row on each side of the rectum, the largest cell in the row being the ventral rectal cells. The dorsal and ventral rectal cells were observed to undergo a considerable increase in size during the fourth stage. The tail was sharper but still terminated in a rounded knob. The excretory system of the third-stage larva consisted of two lateral bands extending from a point just anterior to the excretory pore to the tail; the excretory nucleus was oval and contained a conspicuous nucleolus, it was situated in the posterior third of the oesophagus (Text-fig. 20). The excretory system anteriorly to the excretory nucleus had a cellular structure (Text-fig. 20), being composed of a number of cell-like units, but without apparent nuclei. The genital rudiment was oval and consisted of twelve or more cells (Text-fig. 19). Feline-strain third-stage larvae (length 0·52–0·65 mm.) were found in mouse tissues at 21 and 28 days after infection, they were also found in the intestinal wall of egg-infected cats at 21–35 days after infection and in mouse-infected cats at 13–28 days; they had a length range of 0·47–0·72 mm. Canine-strain third-stage larvae were found in mouse tissues at 8–14 days after infection (length 0·62–0·69 mm.) in the muscles of mouse-infected cats (length 0·75–0·79 mm.), and in the intestinal wall and contents of egg-infected dogs (length 0·49–0·82 mm.).

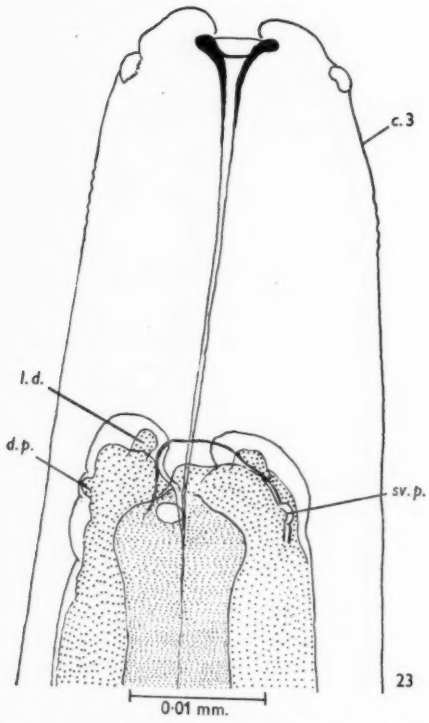
*The third moult* (Text-figs. 21–24)

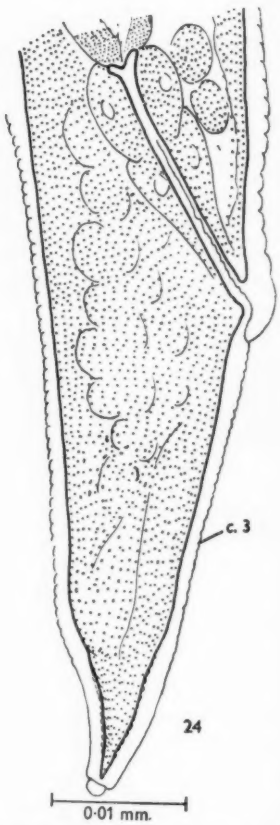
Considerable difficulty was experienced initially in distinguishing between larvae undergoing the second moult and those undergoing the third moult. In egg-infected cats the length of some second-stage larvae overlapped the length of what appeared to be fourth-stage larvae. Hence it was difficult, at first, to differentiate any third larval stage or any third moult in egg-infected cats. Detailed study of moulting larvae in egg-infected cats showed, however, that there were actually two kinds of moulting forms: (1) those in which the larva had a knobbed tail within a knobbed-tailed sheath and the oral region of the larva had no defined lips, i.e. second moults (Text-figs. 10–13); and (2) those in which there was a sharp-tailed larva within a knob-tailed sheath and the larva within the sheath possessed well-defined lips, i.e. third moults (Text-figs. 21–24). The latter were longer than the former, their length ranging from 0·56 to 0·74 mm. The third moult was observed in the intestinal wall of egg-infected cats (C27, C88, C90)—at 3–5 weeks after infection, in a mouse-infected cat (C82) at 5 weeks after infection.

The third moult was characterized by an increased prominence of the cuticular lining of the buccal region, a separation of the cuticle at the anterior and posterior ends of the larva and a cuticular swelling in the anal region. The pointed tail of the fourth-stage larva could be seen within the blunt-tailed sheath of the third stage (Text-fig. 24). Reorganization of the oral region was evident during this



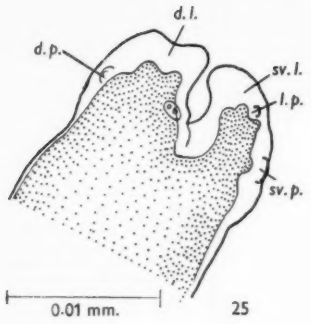

*T. leonina.* Text-fig. 21. Anterior end at third moult (length 0·68 mm.). Text-fig. 22. Anterior end at third moult (length 0·71 mm.). Text-fig. 23. Anterior end at third moult (0·70 mm.). Text-fig. 24. Tail region at third moult (length 0·63 mm.).

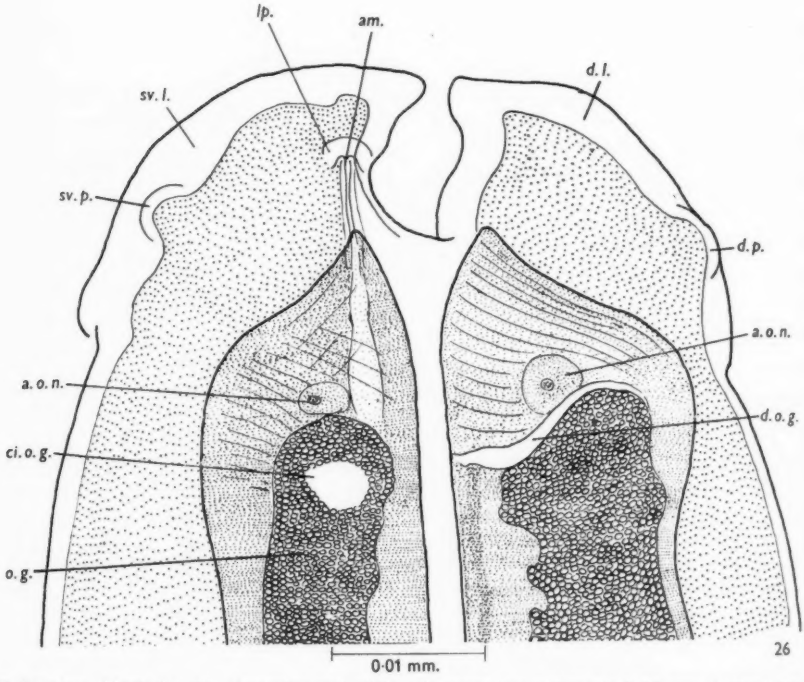
moult; the three lips of the fourth-stage became differentiated as triangular lobes enclosing an oral cavity (Text-figs. 22, 23); moulting glands (?) were present (Text-fig. 22).

*The fourth-stage larva* (Plate VI, figs. 3, 4, 6, 7)

Fourth-stage larvae were observed in the intestinal wall and intestinal contents of cats infected with feline-strain eggs, in cats infected with feline-strain infected mice and canine-strain infected mice, and in dogs infected with feline-strain eggs and canine-strain eggs. The smallest fourth-stage larva observed was 0·56 mm. long. The cuticular sheath of the third stage was still adherent in the tail region. The larva could be readily differentiated from the third-stage larva by the presence of distinct lips constricted from the body, and the sharp dorsally curved tail. A series of larvae with these characteristics was studied, and it was evident that no loosening of the cuticle occurred up to a length of 7·7 mm. Hence it was concluded that this period of growth constituted a single phase of development, i.e. the fourth stage. It was terminated by the fourth moult, after which the adult stage was reached. Apart from growth in length, considerable differentiation of internal structure occurred during the fourth stage. The lips of the fourth-stage larva were similar to those of the adult, but were shallower and less deeply constricted from the body (Text-fig. 26). The labial papillae which were evident in lateral view consisted of two large double papillae on the dorsal lip, and on each subventral lip there was a double papilla resembling those on the dorsal lip and a single lateral papilla situated near to the amphid. The latter consisted of a glandular mass extending into the parenchyma of the subventral lip posteriorly to the level of the anterior oesophageal nucleus (Text-fig. 26); running forwards from this region there was a duct which opened onto the lateral region of the subventral lip. In *en face* view the double papillae appeared to consist of two distinct parts, one with a flat granular pulp and the other with a smaller and more rounded pulp (Text-fig. 27). The pulp of the lips was aggregated into two rounded lobes at the oral margin and in the concave border of each lobe there was a minute granule which probably represents the six internal labial papillae (Text-fig. 27). The oral margin of each lip bore three lancet-like extensions which formed the framework of the oral aperture. Behind these, on each lip, was a short row of peg-like denticles (Text-fig. 27).

The oesophagus was distinctly striated and granular, especially in the anterior part. This was caused by the oesophageal glands. In small fourth-stage larvae they appeared to fill the oesophagus, but as the larvae grew the gland tissue was more compacted. There were three glands, one in each sector of the oesophagus and they extended from the anterior oesophageal nucleus (Text-fig. 26), just behind the lips, to the posterior end of the oesophagus. In the posterior part the granules were scarce, so that the terminal part of the oesophagus was more translucent. The ducts of the subventral glands were not discovered; probably they opened posteriorly as observed in the adult. The duct of the dorsal gland opened into the lumen of the oesophagus at the level of the anterior oesophageal nucleus (Text-fig. 26). In the anterior part of the glands relatively large cistern-like cavities were observed but no lining could be discerned (Text-fig. 26). In the

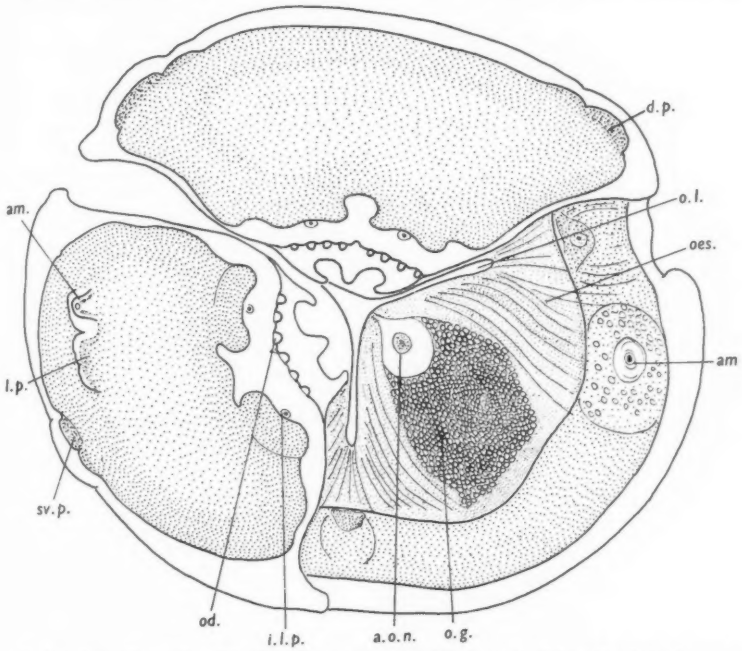



*T. leonina.* Text-fig. 25. Anterior end of fourth-stage larva (length 0·56 mm.). Text-fig. 26. Anterior end of fourth-stage larva (length 3·4 mm.).

terminal part of the oesophagus, there were a number of disk-like hyaline bodies which were regarded as nucleolar particles. In the small larvae they were scattered in a haphazard manner (Text-fig. 28), but as the growth proceeded there appeared an aggregation of granular material, which seemed to congregate around the nucleolar particles and eventually to be confined within a definite membrane (Text-fig. 29). One of these was very large, it acquired an 'S' shape and occupied most of the dorsal sector of the oesophagus (Text-fig. 30). This structure contained several nucleolar particles and was regarded as the nucleus of the dorsal oesophageal

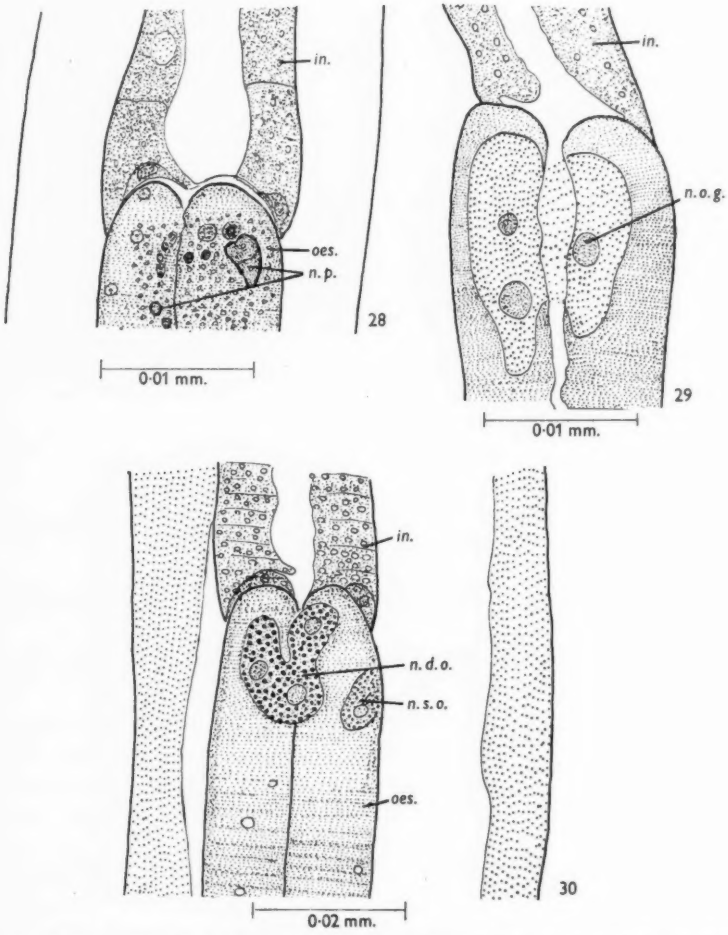
gland. The smaller aggregations were regarded as the nuclei of the subventral glands (Text-fig. 30). At the junction of the oesophagus and the intestine there were two cells which appeared to form a valve-like structure (Text-fig. 28). The cells of the intestine increased in number as growth proceeded and they changed from rectangular to columnar in shape, in older larvae the intestine having a brown tinge. At the junction with the rectum there appeared to be another valve, and




*T. leonina.* Text-fig. 27. *En face* view of anterior end of fourth-stage larva (length about 5 mm.).

just anterior to the valve the cells of the intestine had a conspicuous 'brush' border (Text-fig. 31). The rectum was funnel-shaped, lined with cuticle, and its wall was composed of a number of cells, the anterior ones being elongate. Dorsal to the rectum there was a median row of cells, as observed in third-stage larvae, and ventrally there was a double row (Text-fig. 31). As growth proceeded the anterior cells in the three rows, i.e. the dorsal rectal cell and the two ventral rectal cells, became relatively larger than the others and at the end of the fourth stage they were very conspicuous. There were several ganglion cells in the tail, one triangular cell on the dorsal side of the recto-intestinal junction, a similar cell ventral to the junction, and a ganglion cell in the commissure extending from near the anus to the dorsal body wall (Text-fig. 31). Another group of cells was also defined in the lateral walls of the tail, but as the larva grows the cavity in the tail becomes more capacious and in a focus at which the rectum is defined, these cells are no longer visible. In the centre of the tail cavity there was a stellate ganglion cell in older larvae (Text-fig. 31).

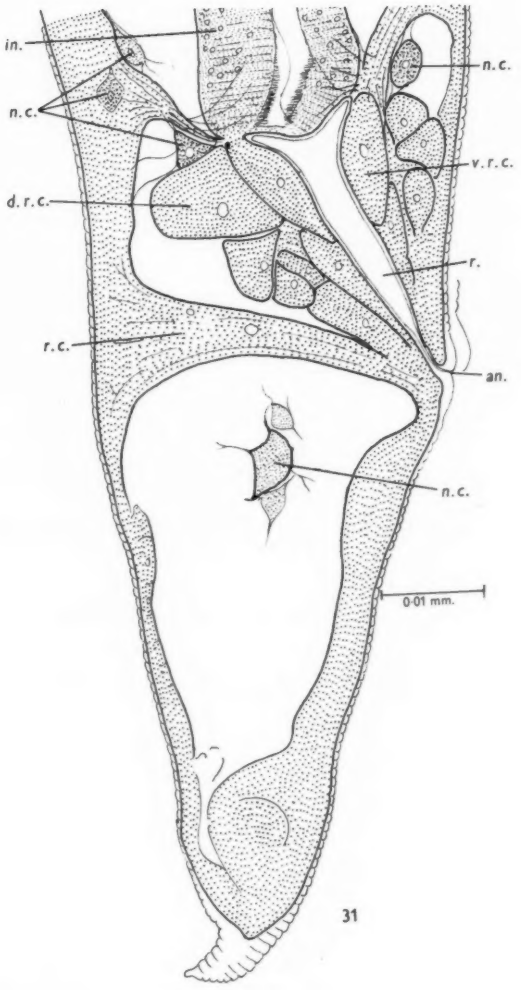
During the fourth stage sexual differentiation became apparent. This consisted of development of the genital rudiment into the gonads and development of the spicules and vagina. The differentiation of the sexes was first evident by the appearance of the genital rudiment. In a larva with a length of 0·75 mm. the genital rudiment had commenced to elongate in an anterior direction (Text-fig. 33) indicating that it would become a female. As growth proceeds the cells of the




*T. leonina*. Text-fig. 28. End of oesophagus of fourth-stage larva (length 0·56 mm.). Text-fig. 29. End of oesophagus of fourth-stage larva (length 0·7 mm.). Text-fig. 30. End of oesophagus of fourth-stage larva (length 1·2 mm.).

genital rudiment evidently increase in number and the anterior prolongation curves down towards a point at the ventral midline where it either forms or meets a bunch of cells (Text-fig. 34). Posteriorly the genital rudiment becomes thicker and divides into two finger-like lobes which extend backwards along the intestine

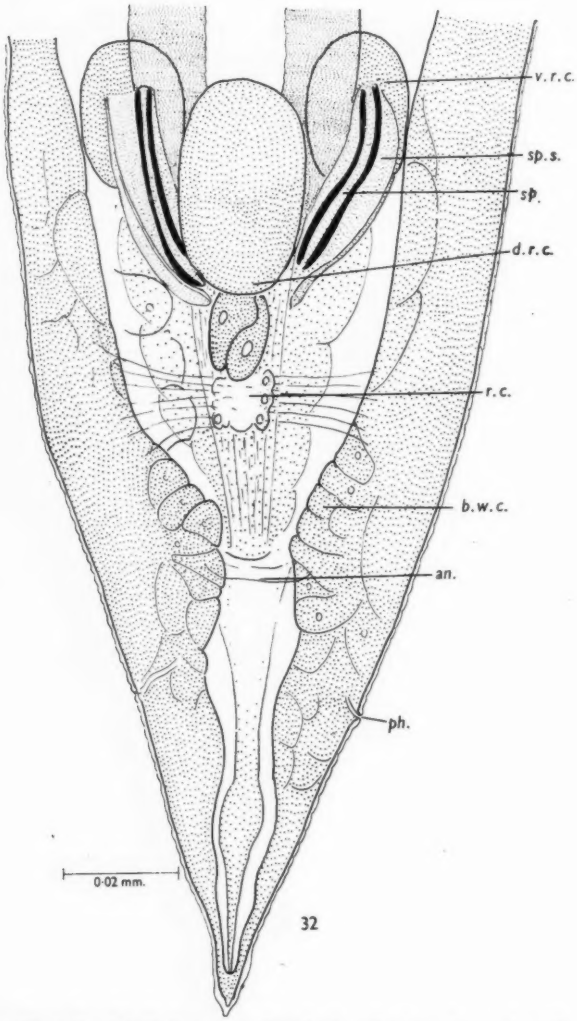
(Text-fig. 35). In larvae about 4 mm. long, the vagina, undivided uterus, and utero-ovarian filaments were defined (Text-fig. 36). The filaments later become coiled around the intestine. At 6 mm. the vagina had a cuticular lining, but the vulva remained closed until the adult stage. In male larvae the development of the genital rudiment seemed to be retarded: it extended as a filament in a posterior




*T. leonina.* Text-fig. 31. Tail region of female fourth-stage larva (length 1·76 mm.).

direction (Text-fig. 37), but its progress was more difficult to follow. At about the same stage of growth an extension from the ventral part of the rectum was observed which probably formed the ejaculatory duct and associated structures. At a length of 2·9 mm. the spicule primordia were visible dorso-lateral to the

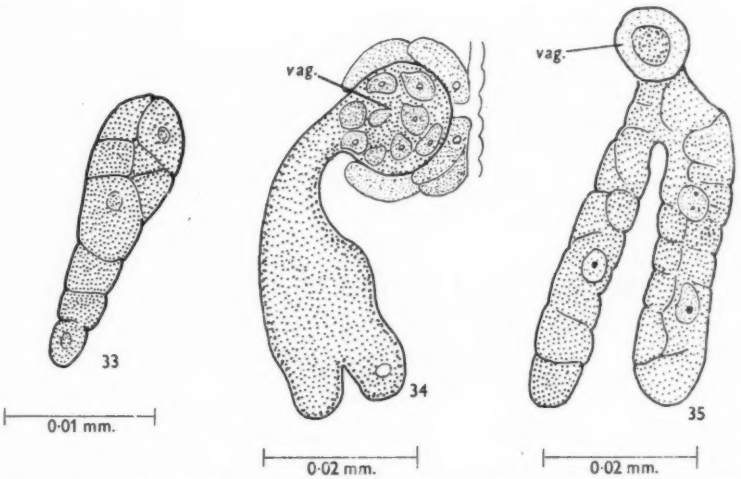
rectum, just behind the dorsal rectal cell. At 4·8 mm. the spicules themselves were observed (Text-fig. 32). Male and female larvae were differentiated also by the shape of the tail. In the female it was relatively long, straight and slender, whereas in the male it was shorter and directed dorsally. None of the caudal papillae were observed in fourth-stage larvae, but in both sexes the phasmids




*T. leonina.* Text-fig. 32. Tail region of male fourth-stage larva (length 4·9 mm.).

were, differentiated on each side of the tail, as pits with a minute canal leading into the parenchyma of the tail (Text-fig. 32). The excretory system appeared to undergo little change in the fourth stage, except that the excretory nucleus moved relatively nearer to the excretory pore. In a larva 2·9 mm. long the nucleus

measured 0·023 mm. in length. There was considerable development of the body musculature and the body cavity became more capacious during the fourth stage. The lateral alae extended along the length of the body, from the nerve ring to the tail. Tables 6 and 8 show measurements of feline- and canine-strain fourth-stage larvae.




*T. leonina.* Text-fig. 33. Genital rudiment of female fourth-stage larva (length 0·75 mm.). Text-fig. 34. Genital rudiment of female fourth-stage larva (length 1·7 mm.). Text-fig. 35. Genital rudiment of female fourth-stage larva (length 2·5 mm.).

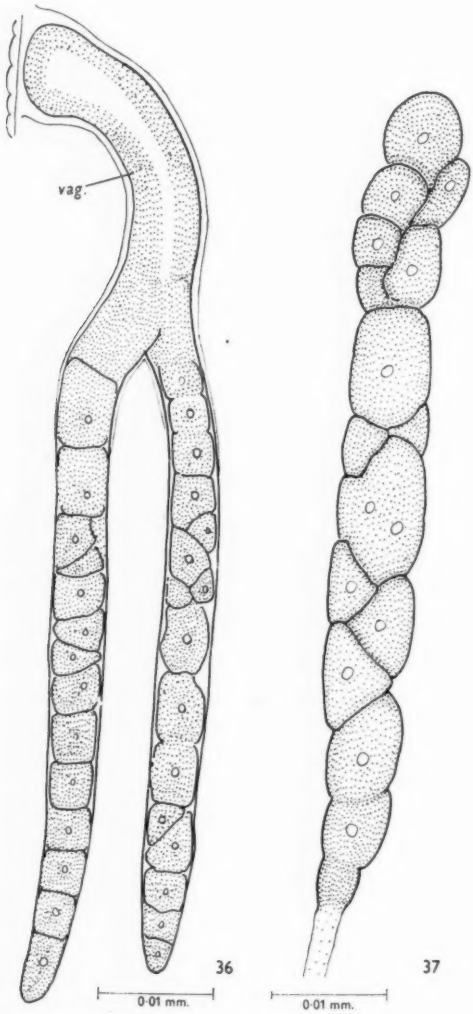
### *The fourth moult* (Pl. VI, fig. 8)

The fourth moult was observed in larvae measuring 7·7–11·7 mm. in length. The cuticle was separated from the adult stage all along the length of the body. A moulting male 7·7 mm. long had about seventeen pairs of pre-anal papillae already defined. The cuticular annulations of the adult stage were each about one-tenth the width of those on the sheath of the fourth stage. A moulting male, 9·2 mm. long, had twenty-six pre-anal papillae. A moulting male, 8·8 mm. long, had spicules 0·192 mm. long. In a moulting female, 11·7 mm. long, the vulva was situated 5·7 mm. from the anterior end.

### *The adult* (Pl. VI, fig. 9)

The adult stage differed from the fourth stage in the larger lips, more deeply constricted from the body and containing elongate extensions of the parenchyma, i.e. the single median internal and the paired external lobes (Text-fig. 38); the latter each bearing the remnant of the internal papilla on its anterior surface. The oral margin of the lips bore denticles which were more numerous, sharper and smaller than those of the fourth stage. The adult also differed in the considerably smaller cuticular annulations, and in the presence of caudal papillae in the male. The nuclei of the oesophageal glands in the adult consisted of an irregularly oval nucleus in each of the subventral sectors (Text-figs. 39, 40), and a very large 'S'

shaped nucleus in the dorsal sector (Text-fig. 39). The latter contain twelve or more nucleolar particles of various sizes. The duct of the dorsal oesophageal gland was situated at the level of the anterior oesophageal nucleus. The ducts of the




*T. leonina.* Text-fig. 36. Genital rudiment of female fourth-stage larva (length 3·4 mm.).
Text-fig. 37. Genital rudiment of male fourth-stage larva (length 2·9 mm.).

subventral glands opened near the gland nuclei. The smallest adult observed, an adult male 8·4 mm. long, possessed all these adult characteristics. On each side of the tail there were a pair of post-anal lateral papillae, a phasmid, and a pair of post-anal subventral papillae; there was also a double papilla on each side of the

anus and thirty pairs of pre-anal papillae. Table 7 shows the measurements of various structures in adult specimens from experimentally infected animals at various stages of growth.

## V. COMPARISON OF FELINE AND CANINE STRAINS

Differentiation of the two strains was studied by comparison of larvae and adults collected from experimentally infected mice, cats and dogs. Comparison was made by measuring certain anatomical features such as length, width, length of

Table 6. *Measurements (mm.) of larvae at various stages of growth (feline strain)*

| | Length | Width | Oeso-phagus | Excretory pore | Tail | Genital rudiment | Oesophagus/ length ratio |
|---|---|---|---|---|---|---|---|
| | | | | 2nd stage | | | |
| 2095 | 0·193 | 0·013 | 0·077 | 0·040 | 0·017 | 0·057 | 0·399 |
| 2085 | 0·388 | 0·025 | 0·110 | 0·056 | 0·027 | 0·109 | 0·284 |
| C86 | 0·434 | 0·022 | 0·138 | 0·062 | 0·033 | 0·123 | 0·318 |
| C86 | 0·549 | 0·024 | 0·165 | 0·068 | 0·036 | 0·163 | 0·301 |
| | | | | 3rd stage | | | |
| 839 | 0·601 | 0·031 | 0·167 | 0·081 | 0·036 | 0·163 | 0·277 |
| C82 | 0·672 | 0·034 | 0·226 | 0·086 | 0·037 | 0·171 | 0·336 |
| C82 | 0·700 | 0·036 | 0·225 | 0·100 | 0·046 | 0·210 | 0·321 |
| | | | | 4th stage | | | |
| C26 | 0·560 | 0·025 | 0·171 | 0·060 | 0·030 | 0·157 | 0·306 |
| C27 | 0·640 | 0·029 | 0·212 | 0·081 | 0·034 | 0·181 | 0·331 |
| C87 | 0·696 | 0·031 | 0·241 | 0·090 | 0·036 | 0·188 | 0·346 |
| C87 | 0·766 | 0·032 | 0·228 | 0·091 | 0·039 | 0·210 | 0·298 |
| C91 | 0·800 | 0·037 | 0·278 | 0·100 | 0·036 | 0·244 | 0·348 |
| C28 | 0·920 | 0·040 | 0·271 | 0·102 | 0·038 | 0·261 | 0·295 |
| C90 | 1·24 | 0·054 | 0·353 | 0·128 | 0·045 | 0·343 | 0·285 |
| C28 | 1·42 | 0·063 | 0·370 | 0·129 | 0·052 | 0·420 | 0·261 |
| | | | | Males | | Spicules | |
| C81 | 1·43 | 0·057 | 0·374 | 0·134 | 0·048 | — | 0·262 |
| C90 | 3·30 | 0·099 | 0·735 | 0·199 | 0·072 | — | 0·223 |
| C35 | 3·77 | 0·126 | 0·759 | 0·183 | 0·087 | 0·018 | 0·201 |
| D55 | 6·8 | 0·152 | 0·910 | 0·235 | 0·100 | 0·072 | 0·134 |
| D55 | 8·6 | 0·193 | 1·070 | 0·280 | 0·084 | 0·101 | 0·124 |
| | | | | Females | | Vulva | |
| C90 | 2·37 | 0·093 | 0·584 | 0·168 | 0·078 | 0·906 | 0·246 |
| D54 | 3·2 | 0·077 | 0·581 | 0·193 | 0·091 | 1·18 | 0·182 |
| C35 | 5·8 | 0·154 | 1·050 | 0·241 | 0·106 | 2·83 | 0·181 |
| C76 | 7·11 | 0·177 | 1·180 | 0·264 | 0·110 | 3·06 | 0·166 |
| C91 | 8·6 | 0·210 | 1·190 | 0·283 | 0·119 | 4·18 | 0·138 |

oesophagus, position of the excretory pore, length of tail, and where present, the length of spicules and the position of the vulva. The only feature which appeared to indicate that a difference existed between the two strains was the oesophagus/total length ratio. In the early stages of growth, the oesophagus/length ratio was higher in feline-strain larvae than in canine-strain larvae; as growth proceeded

this difference became less marked and the ratio itself diminished in both strains (see Tables 6–8).

Adult specimens of *T. leonina* derived from the lynx, arctic fox, red fox, dingo, wolf, and dog, were compared in relation to the body length, the position of the vulva, length of oesophagus, length of spicules, the number and arrangement of caudal papillae, and the morphology of the lips. The only feature which indicated any morphological distinction was the oesophagus/body length ratio. The

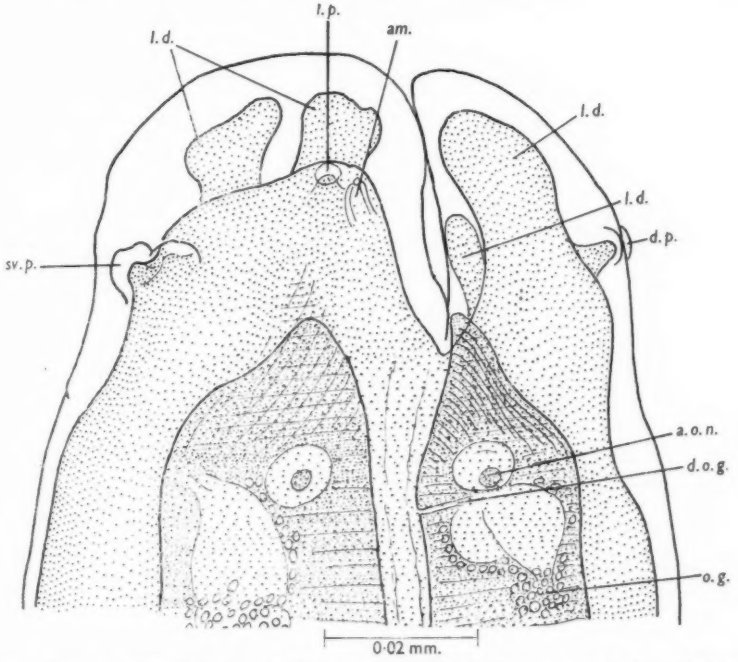
Table 7. *Measurements (mm.) of adults at various stages of growth (feline strain)*

| | Length | Width | Oeso-phagus | Excretory pore | Tail | Vulva | Oesophagus/ length ratio |
|---|---|---|---|---|---|---|---|
| | | | | Females | | | |
| C88 | 10·9 | 0·231 | 1·45 | 0·304 | 0·175 | 4·1 | 0·133 |
| C91 | 16·7 | 0·259 | 1·73 | 0·374 | 0·184 | 8·5 | 0·104 |
| D55 | 19·6 | 0·339 | 1·61 | 0·476 | 0·350 | 8·7 | 0·082 |
| D55 | 23·8 | 0·380 | 1·92 | 0·413 | 0·301 | 10·8 | 0·081 |
| C68 | 46 | 0·560 | 3·55 | 0·885 | 0·451 | 18·5 | 0·077 |
| C68 | 51 | 0·633 | 3·65 | 0·693 | 0·388 | 21·0 | 0·072 |
| | | | | Males | | | |
| | | | | | | Spicules | |
| C88 | 8·4 | 0·206 | 1·19 | 0·301 | 0·100 | 0·239 | 0·142 |
| D54 | 10·3 | 0·217 | 1·37 | 0·350 | 0·126 | — | 0·133 |
| C35 | 19·5 | 0·331 | 2·07 | 0·364 | 0·112 | 0·448 | 0·106 |
| D55 | 19·6 | 0·332 | 1·74 | 0·385 | 0·138 | — | 0·089 |
| C35 | 40 | 0·580 | 3·48 | 0·588 | 0·210 | 0·940 | 0·088 |
| C65 | 51 | 0·580 | 3·91 | 0·759 | 0·196 | 1·050 | 0·077 |

Table 8. *Measurement (mm.) of larvae at various stages of growth (canine strain)*

| | Length | Width | Oeso-phagus | Excretory pore | Tail | Genital rudiment | Oesophagus/ length ratio |
|---|---|---|---|---|---|---|---|
| | | | | 2nd stage | | | |
| 2056 | 0·216 | 0·017 | 0·068 | 0·035 | 0·037 | 0·057 | 0·315 |
| 2065 | 0·350 | 0·025 | 0·090 | 0·061 | 0·025 | 0·103 | 0·257 |
| 2062 | 0·553 | 0·031 | 0·149 | 0·072 | 0·030 | 0·157 | 0·269 |
| | | | | 3rd stage | | | |
| C84 | 0·770 | 0·040 | 0·178 | 0·095 | 0·039 | 0·223 | 0·231 |
| | | | | 4th stage | | | |
| C85 | 0·753 | 0·037 | 0·187 | 0·093 | 0·051 | 0·241 | 0·248 |
| C84 | 0·770 | 0·037 | 0·210 | 0·094 | 0·045 | 0·272 | 0·273 |
| C84 | 0·910 | 0·042 | 0·212 | 0·122 | 0·043 | 0·282 | 0·233 |
| C84 | 0·990 | 0·044 | 0·222 | 0·138 | 0·045 | 0·286 | 0·224 |
| | | | | Males | | | |
| | | | | | | Spicules | |
| C84 | 2·6 | 0·099 | 0·550 | 0·177 | 0·072 | — | 0·212 |
| C84 | 3·2 | 0·093 | 0·602 | 0·194 | 0·080 | — | 0·188 |
| C84 | 8·8 | 0·210 | 1·09 | 0·301 | 0·109 | 0·220 | 0·124 |
| | | | | Females | | | |
| | | | | | | Vulva | |
| C58 | 1·6 | 0·074 | 0·374 | 0·153 | 0·051 | 0·540 | 0·234 |
| C84 | 2·5 | 0·103 | 0·490 | 0·178 | 0·081 | 0·910 | 0·196 |
| D58 | 5·9 | — | 0·791 | 0·227 | 0·122 | 1·95 | 0·134 |

oesophagus of the feline strain attained a greater length in relation to the total body length than the specimens from Canidae, thus confirming the observations made on the larvae from experimental animals (see Table 9).




*T. leonina*. Text-fig. 38. Subventral view of anterior end of young adult (length 8·4 mm.).

Table 9. *Oesophagus/length ratio of specimens* of* Toxascaris leonina *from various hosts*

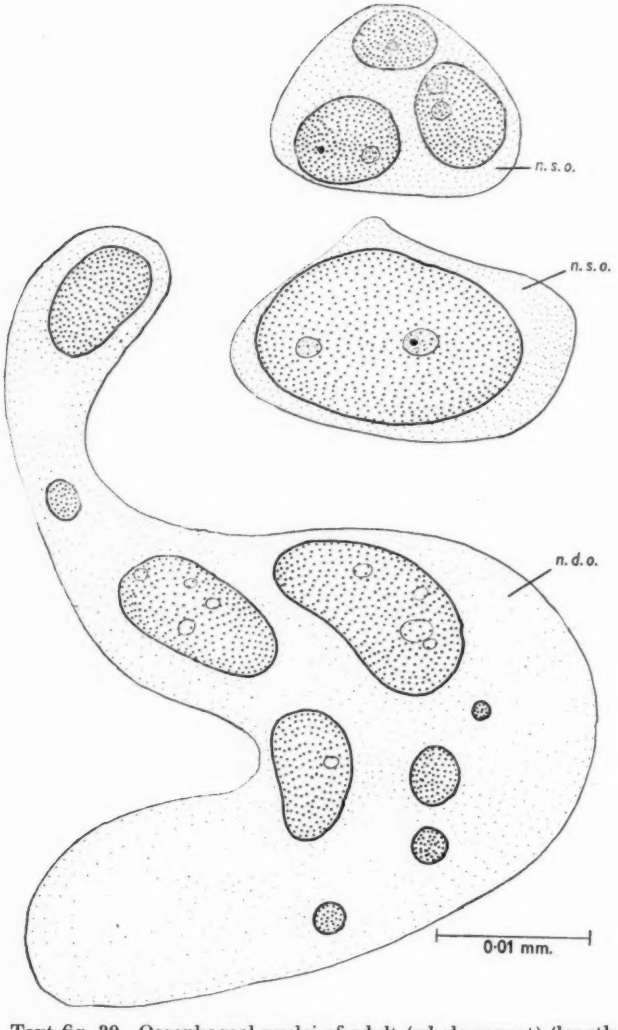
| Host | No. of specimens examined | Oesophagus/length ratio | |
|---|---|---|---|
| | | Range | Average |
| *Lynx canadensis* | 17 | 0·06–0·11 | 0·09 |
| *Canis familiaris* | 12 | 0·04–0·07 | 0·06 |
| *C. dingo* | 2 | 0·065–0·075 | — |
| *C. lupus* | 19 | 0·03–0·09 | 0·07 |
| *Vulpes fulva* | 11 | 0·04–0·06 | 0·05 |
| *Alopex lagopus* | 10 | 0·05–0·08 | 0·06 |

* Method of fixation and condition at time of collection was unknown.

## VI. DISCUSSION

In comparing their life histories, it must be pointed out that, though *Toxascaris leonina* and *Toxocara* spp. resemble one another superficially and are frequently associated together in the same host, they are, nevertheless, distant relations. Among the structural features of *Toxascaris leonina* which differentiate this species from *Toxocara canis* are the extension of the subventral glands to the

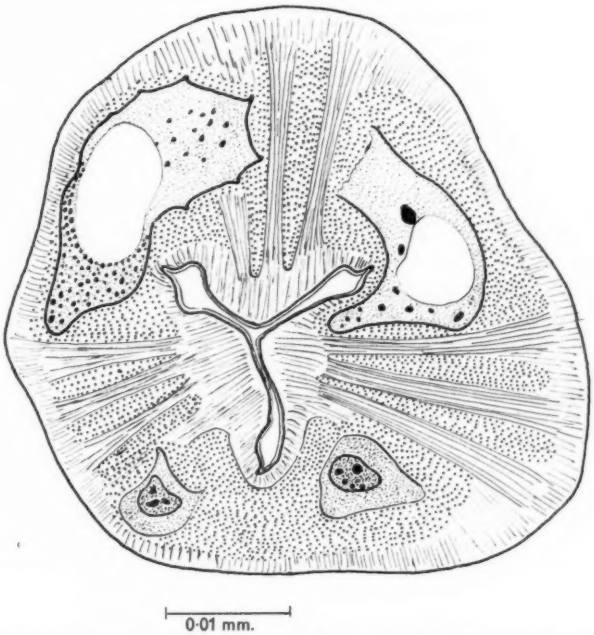
anterior end of the oesophagus, the retention of the dorsal nucleus in the dorsal half of the oesophagus, the absence of any demarcated ventricular region at the posterior end of the oesophagus, the non-alate spicules, the non-probular tail of the male and the more distinctly double papillae on the dorsal and subventral lips. In all these characters *Toxascaris leonina* shows a closer structural affinity with the




*T. leonina*. Text-fig. 39. Oesophageal nuclei of adult (whole mount) (length 8·4 mm.).

genus *Ascaris* than with *Toxocara*. The presence of 'cervical' alae appears to be a more superficial character, because they do not appear until the adult stage (these should not be identified with 'lateral' alae which are probably possessed by all ascaridoid larvae, but are not present in the mature adult).

It is therefore to be expected that, in its development, *Toxascaris leonina* is likely more closely to resemble that of *Ascaris suum* and *A. devosi* than that of *Toxocara canis* and *T. cati*. On the other hand, being a parasite of the same hosts as the latter two species, it has been subjected to the same influences in adapting its life history to the habits of its hosts. Hence it is also likely that certain similarities may be found to exist between the life history of *Toxascaris leonina*, *Toxocara canis* and *T. cati*, but that such similarities denote a parallel evolutionary trend rather than a close relationship.




*T. leonina.* Text-fig. 40. Oesophageal nuclei of adult (stained section).

On hatching, the second-stage larvae are slightly smaller than those of *Ascaris suum* and *A. devosi* as observed by Roberts (1934) and Sprent (1953*a*) respectively, and considerably smaller than those of *Toxocara* spp. (Sprent, 1956, 1958). They resemble *Ascaris suum* and *A. devosi* in that the second-stage larvae undergo a period of growth, attaining about the same length as *A. suum*, but a shorter length than that attained by second-stage larvae of *A. devosi*. In contrast, the second-stage larvae of *Toxocara* spp. undergo little or no growth. The third-stage larva of *Toxascaris leonina* is a resting stage and in this respect resembles the third-stage larvae of *Ascaris devosi*, although it is considerably smaller than the latter, so that the third moult occurs at a considerably shorter length. In contrast, the third stage in *Toxocara* spp. is a period of active growth. The onset of the fourth stage in *Toxascaris leonina* occurred at a shorter length than in the other species, but its termination at the fourth moult occurred at about 12 mm., compared with

about 16 mm. for *Ascaris devosi* and about 16–20 mm. for *A. suum*. Again, in contrast, the fourth moult of the *Toxocara* spp. occurred at a considerably shorter length, i.e. about 4–7 mm.

It is evident therefore that with regard to growth, the development of *Toxascaris leonina* more closely resembles that of *Ascaris* spp. than that of *Toxocara* spp. With regard to morphology, there was no indication that the above-mentioned features which *Toxascaris leonina* shares with *Ascaris* spp. are secondary characters. As features such as the labial papillae, oesophageal glands, etc., become defined in the larvae of *T. leonina*, their differential characters are evident from the beginning, and can be differentiated from the corresponding structures of *Toxocara* larvae.

On the other hand, the migratory behaviour of the larvae of *T. leonina* is quite different from any of the above-mentioned species. Further work is required to elucidate the migratory pathway taken by these larvae when they leave the intestinal wall of mice and proceed to invade the somatic tissues. The scarcity of liver lesions indicates that this organ is not traversed by the larvae, but the occurrence of lung lesions indicates that the larvae reach the lungs in relatively large numbers during their migration. Furthermore, the occurrence of encapsulated larvae in the neck and head region indicates a distribution via the arterial bloodstream. The frequent occurrence of larvae encapsulated in the retroperitoneal and perirectal tissues, and abdominal wall, suggests that most of the larvae migrate from the intestinal wall into the abdominal cavity and thence pass directly to such tissues. It seems likely that their appearance in other parts of the body might be explained by penetration of some of the larvae into the mesenteric lymph vessels and their subsequent migration via the thoracic duct into the circulation.

Their migration and encapsulation in mouse tissues and the successful infection of the final hosts through ingestion of infected tissues indicates that intermediate hosts are utilized in the life history. It would appear likely that this might be the most usual method of infection where the larger predatory hosts are involved.

On the other hand, it is evident that intermediate hosts are not necessary, and that the whole larval development can proceed in the intestinal wall of the final host. The ascaridoids of mammals are remarkable in their evident capacity for adaptation to the nutritional habits of their particular host: their life histories are of diverse patterns and this has been discussed in relation to evolutionary trends within the group (Sprent, 1954). The wide range of hosts of *T. leonina* means that this parasite has had to adapt its life history to a wide range of nutritional habits. *T. oxocara cati* has had to adapt its life history to a number of hosts, but these hosts are all felines and all have a predatory habit, so that, as one might expect, a wide range of intermediate hosts is utilized by this species, but infection through ingestion of eggs is probably rare. *T. canis*, on the other hand, has had to adapt its life history to the nutritional habits of canines, i.e. in many instances to scavenging. Hence this species has evidently tended to dispense with the intermediate host and adopt the migratory behaviour of the larvae towards prenatal infection, through ingestion of eggs by the mother.

In contrast, *Toxascaris leonina* occurs in both felines and canines, and thus has had to retain a high degree of adaptability in its life history, so that whether the

host is a scavenger or a predator, infection can occur. The situation appears to have been met by delaying the migration of the second-stage larvae, so that they do not enter the blood vessels of the intestine and thus do not undergo any somatic migration. In this way, there is a period of growth in the intestinal wall, which allows for further development, whether the larvae are in the final host or in an intermediate host. If the larvae are in the intestinal wall of the final host, the third stage is unnecessary, so that the second and third moults follow one another rapidly, and the fourth-stage larvae proceed to the intestine for further development.

On the other hand, if the larvae are in the intestinal wall of an animal which is not a suitable final host, the period of growth is terminated by a migration of the second-stage larvae in the opposite direction, i.e. towards the abdominal cavity and thence via the lymphatics to various somatic tissues. It is probable that when the larva has come to rest the second moult is completed. In these circumstances, the third-stage larva is necessary, for it represents a resting stage, during which the larvae remain encapsulated in various tissues until the animal is ingested by a predatory host.

As the life histories of the various ascaridoids are revealed, it becomes evident that the migratory patterns of the larvae are of wide variety and of considerable significance in determining the mode of infection. It has become evident that each ascaridoid exhibits particular and characteristic migratory patterns, but the biological factors which determine and influence the direction of migration constitute an unexplored and possibly a most significant field of investigation.

## VII. SUMMARY

1. The nomenclature and host specificity of *Toxascaris leonina* is reviewed and the conclusion reached that this parasite occurs in a wide range of feline and canine hosts. Notice is given of application to the International Commission on Zoological Nomenclature, for the suppression of *Ascaris leptoptera* Rudolphi 1809 and *A. microptera* Rudolphi, 1819, which appear to be senior synonyms of *Toxascaris leonina*. The synonyms and hosts of *T. leonina* are tabulated.

2. The distribution and growth of the larvae of *T. leonina* in experimentally infected mice, cats, and dogs is reported. A feline and a canine strain were used for these infections and infection of cats and dogs was produced both by feeding the eggs containing second-stage larvae and by feeding mouse tissues containing third-stage larvae.

3. Distribution of larvae in mice indicated that they remain in the intestinal wall for about a week and then migrate into the somatic tissues and complete the second moult. It appeared that this migration was by direct penetration into the abdominal cavity and thence mainly into adjacent tissues; it seemed likely that further migration may occur, possibly via the lymphatics into the systemic circulation. Macroscopic and microscopic observations on the infected mice are described.

4. No difference was observed in the migratory behaviour and distribution of the larvae of canine and feline strains, but the former were observed to grow to a length of about 0·6–0·7 mm. and to complete the second moult by 9–10 days, whereas the feline strain grew to a length of about 0·5–0·65 mm. and the second

moult was completed between 2 and 3 weeks after infection. A small number of wolf-strain and fox-strain infections showed the length of the larvae to be similar to the dog strain. Mice harbouring third-stage larvae in the tissues were infective to cats 1–5 months after infection with eggs.

5. In cats infected directly by feeding eggs of the feline strain, the second-stage larvae were found in the intestinal wall for 14 days and underwent the second moult at 0·42–0·56 mm. The third stage was brief and was followed by the third moult which was observed at 3–5 weeks after infection, but probably occurred at 2 weeks. The length of moulting third-stage larvae was 0·56–0·74 mm., indicating very little growth in the third stage. Fourth-stage larvae were observed at 14 days. Considerable growth occurred during this stage, the larvae reaching a length of about 8 mm. The fourth moult was observed at 6 weeks at a length of 7·7 mm.; although adults were present at 28 days. Eggs first appeared at 74 days after infection. Three cats infected with canine-strain eggs were negative.

6. Cats were infected by feeding feline-strain infected mice and by canine-strain infected mice and in both series development to the fourth stage was observed; in the latter case the fourth moult was observed.

7. Dogs were infected by feeding eggs of the feline strain and the canine strain. Development to the adult stage was observed in the former and to the fourth stage in the latter. One dog was infected by feeding a mouse infected with the canine strain. Eggs appeared in the faeces at 56 days after infection of this dog.

8. As the canine and feline strains of *T. leonina* were observed to grow to the adult stage in both cats and dogs, it was concluded that these two strains constituted a single species, i.e. *T. leonina*.

9. A description is given of the morphology of the larval stages. Apart from the slightly greater length attained by the third-stage larvae of the canine strain, the only morphological difference between the two strains was the relatively longer oesophagus of the feline strain. This difference became less evident with increase in total length.

10. The length of the larval stages was as follows: first stage, 0·19–0·23 mm.; second stage, 0·19–0·65 mm.; third stage, 0·47–0·72 mm. (feline strain), 0·49–0·82 mm. (canine strain); fourth stage, 0·56–11·7 mm.; adult stage, 8·4–over 100 mm.

11. A number of adult specimens, derived from lynx, red fox, arctic fox, wolf, dingo and dog were examined with regard to the morphology of the lips, caudal papillae, position of vulva, length of spicules and oesophagus/length ratio. Apart from a slightly greater oesophagus/length ratio in the specimens from the lynx, no differences were detected. It was concluded that these observations confirmed those on the larvae, that there is a single species occurring in both felines and canines, although these differences may indicate the convergence of two originally distinct species.

12. The life history and development of *T. leonina* were compared with those of *Ascaris* spp. and *Toxocara* spp. It was concluded that they indicated a closer affinity to the former than the latter.

13. The migratory behaviour of the larvae of *Toxascaris leonina* was discussed,

in relation to adaptation to infection in both Canidae and Felidae. It was suggested that delay in migration of second-stage larvae from the intestinal wall is an adaptation to a range of hosts which includes scavengers and predators. If the larvae, as a result of ingestion of eggs, develop in the intestinal wall of a suitable final host, they migrate towards the intestine, but if in the intestinal wall of an animal unsuitable as a final host, they migrate towards the abdominal cavity and, becoming encapsulated in various tissues, await ingestion by a predator.

The writer's thanks are due to his wife for rearing many of the kittens and puppies used in these experiments, and to Mr J. J. Mines for his able technical assistance. The writer also wishes to acknowledge the help in obtaining certain references that he has received from Professor R. T. Leiper, F.R.S., and from Mr Allen McIntosh of the Agricultural Research Service of the United States Department of Agriculture. The work was supported by a research grant from the University of Queensland.

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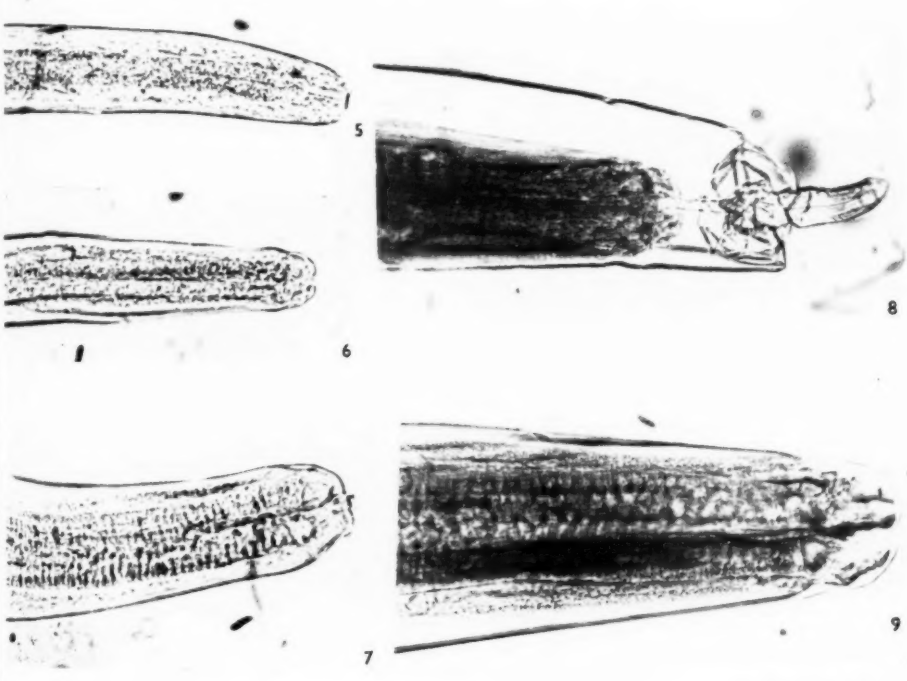


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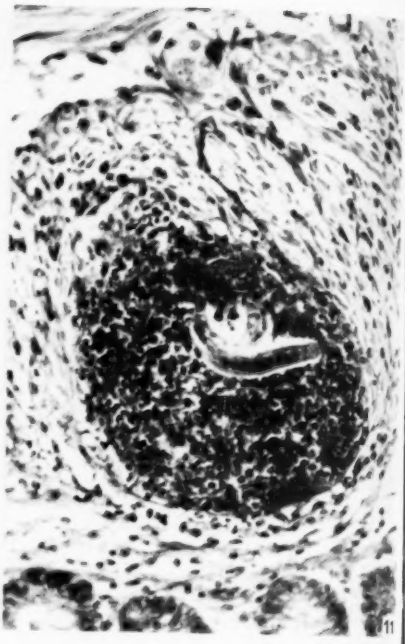

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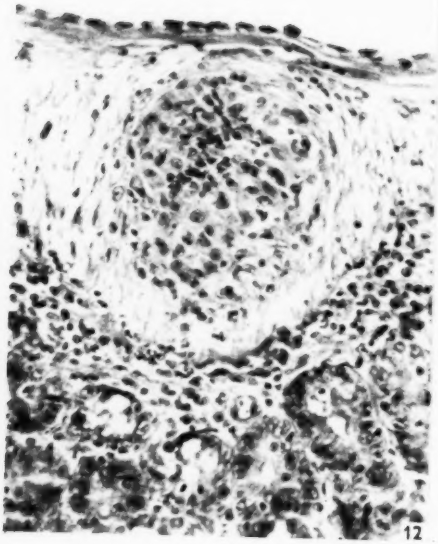

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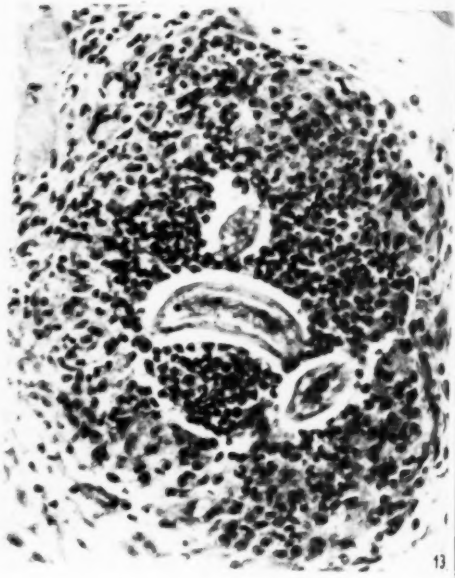

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## EXPLANATION OF PLATES VI AND VII

### Plate VI

Fig. 1. Second-stage larva. × 500.
Fig. 2. Third-stage larva. × 300.
Fig. 3. Fourth-stage larva. × 150.
Fig. 4. Fourth-stage larva. × 150.
Fig. 5. Anterior end of third-stage larva. × 500 (length 0·65 mm.).
Fig. 6. Anterior end of fourth-stage larva. × 500 (length 0·75 mm.).
Fig. 7. Anterior end of fourth-stage larva. × 500 (length 1·75 mm.).
Fig. 8. Anterior end at fourth moult. × 150 (length 9·2 mm.).
Fig. 9. Anterior end of adult. × 300 (length 8·4 mm.).

### Plate VII

Fig. 10. Intestinal wall of mouse about 24 hr. after infection with eggs of *Toxascaris leonina*, showing cell clusters in submucosa. × 44.
Fig. 11. Larva (second stage) of *T. leonina* in granulomatous cluster 2 days after infection. × 300.
Fig. 12. Granuloma from which larva has escaped; 6 days after infection. × 300.
Fig. 13. Larva (third stage) in granulomatous cluster in heart muscle of mouse at 13 days after infection. × 300.

## LIST OF ABBREVIATIONS

| | | | |
|---|---|---|---|
| *am.* | amphid | *n.c.* | nerve cell |
| *an.* | anus | *n.d.o.* | nucleus of dorsal oesophageal gland |
| *a.o.n.* | anterior oesophageal nucleus | *n.o.g.* | nucleus of oesophageal gland |
| *b.w.c.* | body wall cells | *n.p.* | nucleolar particles of oesophagus |
| *c.f.* | cuticular flange | *n.s.o.* | nucleus of subventral oesophageal gland |
| *ci.o.g.* | cistern of the oesophageal gland | *n.r.* | nerve ring |
| *c.2.* | cuticle of second-stage larva | *oes.* | oesophagus |
| *c.3.* | cuticle of third-stage larva | *o.d.* | oral denticles |
| *d.l.* | dorsal lip | *o.g.* | oesophageal gland |
| *d.o.g.* | duct of oesophageal gland | *o.l.* | lumen of oesophagus |
| *d.p.* | dorsal labial papilla | *o.p.* | oral prolongation |
| *d.r.c.* | dorsal rectal cell | *ph.* | phasmid |
| *e.n.* | nucleus of excretory cell | *r.* | rectum |
| *e.p.* | excretory pore | *r.c.* | rectal commissure |
| *e.s.* | excretory system | *rh.* | cuticular thickening of lips |
| *i.l.p.* | internal labial papilla | *sp.* | spicules |
| *in.* | intestine | *sp.s.* | spicule sheath |
| *l.* | lips | *sv.l.* | subventral lip |
| *l.d.* | labial digitation | *sv.p.* | subventral lateral papilla |
| *l.p.* | lateral labial papilla | *v.r.c.* | ventral rectal cell |
| *m.* | mouth opening | *vag.* | vagina |
| *m.g.* | moulting gland | | |

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# THE ATTACHMENT OF EGGS OF A MITE *EPIDERMOPTES BILOBATUS* TO THE SKIN OF ITS HOST

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(With 3 Figures in the Text)

Certain species of mites and insects live permanently in the hair coat of mammals and feather coats of birds. The majority lay eggs which are attached either to hairs or feathers, as with lice, or rest on the surface of the skin, as with certain

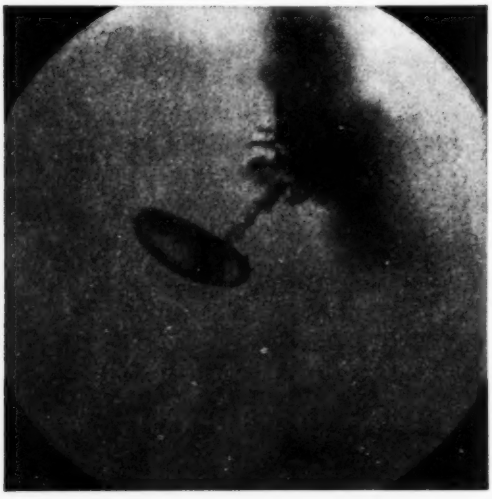

Fig. 1. A photograph of an egg attached to the skin of a turkey poult.

Fig. 2

Fig. 3

Fig. 2. The attachment to the skin.
Fig. 3. The attachment of the stalk to the posterior pole of the egg.

mange mites. However, there appears to be no record in the literature of the eggs of any permanent ectoparasite of birds or mammals which are attached to the skin of its host by means of a distinct stalk. In Fig. 1 may be seen a photograph of the egg of the mite *Epidermoptes bilobatus* Rivolta, 1876 attached to the skin of a turkey poult. Each egg was attached to the skin by a distinct strap-like stalk which held the egg quite clear of the skin. The manner of attachment of this stalk to the posterior pole of the egg was constant (Fig. 2), in contrast to the attachment to the skin (Fig. 3).

Mr H. Womersley of the South Australian Museum kindly confirmed the identification of the mite.

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# A COMPARATIVE STUDY OF THE LIFE HISTORIES OF *NEMATODIRUS BATTUS* AND *N. FILICOLLIS*, NEMATODE PARASITES OF SHEEP

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(With 13 Figures in the Text)

Three species of the genus *Nematodirus*, nematode parasites of the small intestine, have been described from sheep in Britain. *Nematodirus filicollis* was first described by Rudolphi (1802) under the name *Ascaris filicollis*, and was made the type species of the genus *Nematodirus* proposed by Ransom (1907). *Strongylus spathiger* (Railliet, 1896) was placed in this genus and renamed *Nematodirus spathiger* by Railliet & Henry (1909). The genus was reviewed by May (1920), who gave brief descriptions of *N. spathiger* and *N. filicollis*, and noted that these two species could only be differentiated on the characters of the males, the females being indistinguishable. Boulenger (1915) described and figured the adults of *N. filicollis*. The third species, *N. battus*, was described by Crofton & Thomas (1954), who found that this species could be differentiated on the characters of both the male and the female.

The distribution of *N. filicollis* and *N. spathiger* is world-wide, but *N. battus* has so far only been reported from Britain (including Northern Ireland). Little evidence is available about the distribution of the three species in Britain, but *N. spathiger* appears to be relatively rare in northern England, where *N. battus* is the predominant species, with *N. filicollis* present to a lesser extent.

In south-west England Crofton (1957) found mainly *N. filicollis*, with small numbers of *N. spathiger*, and recorded *N. battus* on only one occasion.

The eggs of *N. filicollis* and *N. spathiger* were described and differentiated by Tetley (1941), and by Kates & Shorb (1943). Kates & Turner (1955) gave a detailed description of the life history of *N. spathiger* and figured the larval stages. The life history of this species is typical of the genus, larval development occurring within the egg, the third-stage larva being the only free living stage. The infective larva of this species was described by Mönnig (1931) and by Dikmans & Andrews (1933).

Studies on the development of *N. filicollis* have been limited. Ransom (1911), Railliet & Henry (1912), and Maupas & Seurat (1913), noted that larval development took place within the eggs, two moults occurring before the larva hatched, and the latter authors figured the newly hatched larva. Boulenger (1915) described briefly the development of the larval stages of *N. filicollis* and noted that development was slow compared with that of other trichostrongylid nematodes. This author gave a detailed description and figures of the infective larva and described immature stages from naturally infected sheep. Thomas (1957) compared the infective larvae of *N. spathiger*, *N. filicollis* and *N. battus*, and concluded that the

larva described by Boulenger (1915), was that of *N. spathiger* and not *N. filicollis*. The life history of *N. battus* has not been previously described.

The purpose of this paper is to describe the life history of *N. battus*, with a comparative account of the life history of *N. filicollis*, and to relate the pathogenicity of these species to the development of the parasitic stages.

## MATERIAL AND METHODS

For the preliminary studies of eggs and larvae, material was obtained by the dissection of fresh female worms, as described previously (Thomas, 1957). In later work, once it was established that the eggs of *N. filicollis* and *N. battus* could be reliably differentiated, and that *N. spathiger* was so rare that it could be ignored, eggs were collected from the faeces of lambs with heavy natural infections. Canvas bags were attached to the hindquarters of wether lambs and left on for 24 hr. In this way it was possible to collect a large amount of fresh faeces, uncontaminated by soil or other material.

Several authors (Herlich, 1954; Seghetti, 1955) have reported that culture of *Nematodirus* eggs by the usual method of faeces-charcoal culture gives very poor results, and this was confirmed in early culture work. The lack of success with faeces-charcoal is probably due to the very slow development of *Nematodirus* eggs, the oxygen content of the medium being exhausted before development has proceeded very far, further diffusion of oxygen being too slow to allow development to continue. For this reason it was found necessary to culture the eggs in clean water.

To do this the faeces were thoroughly broken down by mixing with water, and then washed through sieves of 100 and 400 mesh (152 and 37$\mu$ mesh aperture). *Nematodirus* eggs were collected in the latter sieve, together with a considerable amount of vegetable matter, from which they were subsequently separated by centrifugation in saturated salt solution. The eggs were pipetted from the surface of the salt solution, washed, and cultured in water in Petri dishes at 21° C. For culture on a large scale the eggs were incubated in a water-bath fitted with a combined heater–thermostat–stirrer unit. A similar technique for the recovery and culture of *N. spathiger* eggs has recently been described by Seghetti (1955).

For comparative purposes a small number of eggs of *N. spathiger* were obtained by flotation from sheep faeces collected in the Channel Islands, where this species appears to be more common. The eggs were identified on morphology and size measurements and were cultured in water as shown above.

For the study of the parasitic stages, infective larvae were administered to lambs of approximately 6 weeks old which had been reared free from infection. Four lambs each received, orally, 50,000 infective larvae in 10 c.c. of water, each dose consisting of 95 % *N. battus* and 5 % *N. filicollis* larvae. The lambs were slaughtered at 5-, 8-, 12- and 15-day intervals after infection. Immediately after slaughter, the small intestine was tied off and removed. Three sections, each 1 in. in length, were taken at 5, 10 and 15 ft., respectively from the pylorus and were placed in 10 % formol saline. Serial sections were made from these portions

and stained with haematoxylin and eosin for histological study. A further 1–2 in. portion from the first 10 ft. of intestine was also removed, opened, and examined in warm physiological saline, to study the living parasites *in situ*. The rest of the intestine was then opened, the contents removed and the parasites recovered by sieving and preserved in formalin, and counted by a dilution technique. This procedure was based on that used by Kates & Turner (1955) in the investigation of the life history of *N. spathiger*.

Because of the preponderance of *N. battus* in the area, it was extremely difficult to obtain pure cultures of *N. filicollis*, and for this reason the parasitic stages of *N. filicollis* were recovered from the four lambs previously described, which had been given a mixed infection. As in these lambs the differentiation of larval stages in the histological examination was not possible, it could only be concluded that these would be predominantly or entirely *N. battus*. In order, therefore, to confirm that the histological picture was similar for both species, a single lamb was infected with a dose of 20,000 infective larvae of *N. filicollis*, this being the only pure culture available, and this lamb was slaughtered 8 days after infection.

Drawings and measurements were made from temporary mounts in water, the eggs being mounted under raised cover-slips to prevent distortion, and drawings being made with the aid of a camera lucida.

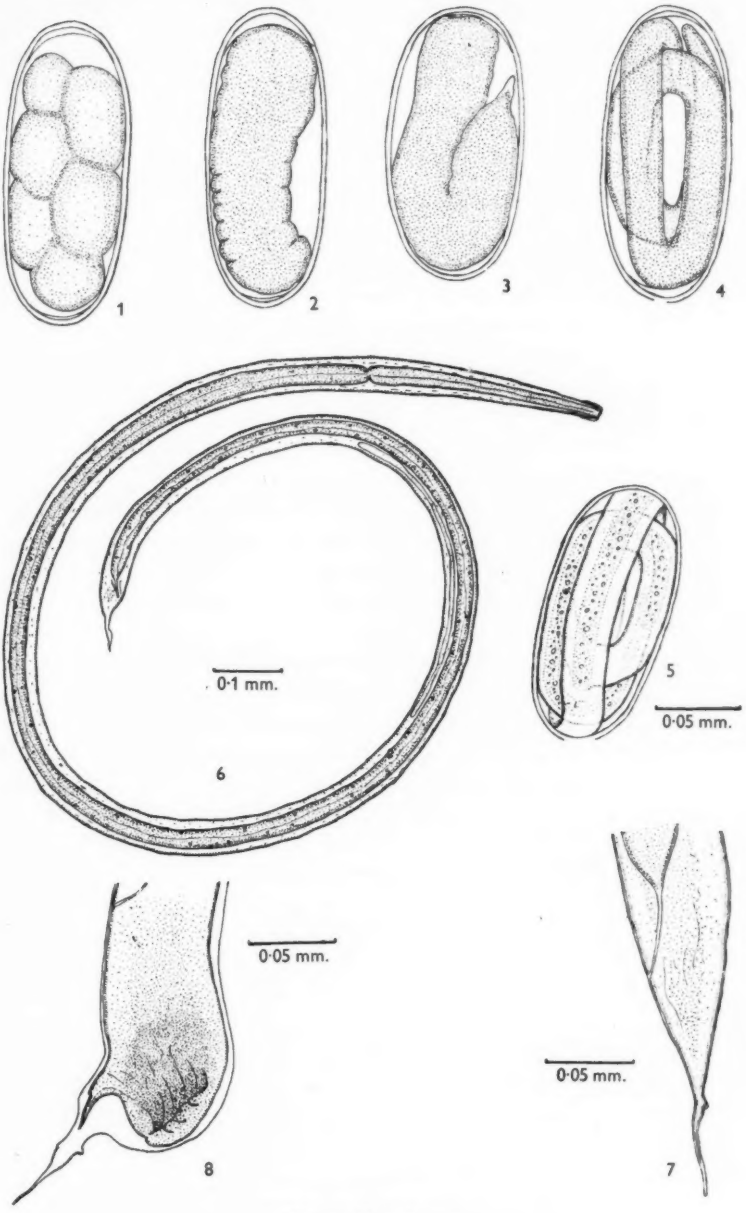
## RESULTS

### *Nematodirus spathiger*

The egg of *N. spathiger* has been described by Tetley (1941) and Kates & Shorb (1943), and those obtained in this work closely resembled the descriptions given by these authors. At 21° C. the eggs developed to the third larval stage in 18–22 days, the developmental stages being as described by Kates & Turner (1955). Fully developed larvae hatched readily at temperatures between 21 and 28° C. and at the latter temperature all the larvae hatched within a few days. Only a small number of larvae were available, but these corresponded closely with the descriptions of Mönnig (1931) and Dikmans & Andrews (1933). Owing to the scarcity of material the parasitic stages could not be investigated, but these have been adequately described by Kates & Turner (1955).

### *Nematodirus battus*

*Preparasitic stages.* The egg of *N. battus* is typical of the genus in being considerably larger than the eggs of other common gastro-intestinal nematodes. Mean measurements for 100 eggs, taken from the ovejector region of female worms, were $164 \times 72\mu$, the range in length being 152–182$\mu$, and in width 67–77$\mu$. The egg is very rounded at the ends, with parallel sides (Fig. 1). The shell has a uniform thickness of 3$\mu$, and the egg, when taken from the maternal uterus, is clear and colourless. Eggs recovered from faeces, however, are markedly brown, the colour being apparently due to some change undergone during passage down the intestine. The egg, when it is passed in the faeces, normally contains 7–8 large, dark, granular cells and the vitelline membrane is usually visible at the poles.




*Nematodirus battus*

Figs. 1–8. Fig. 1. Undeveloped egg. Fig. 2. Late morula stage. Fig. 3. Tadpole stage. Fig. 4. First-stage larva. Fig. 5. Third-stage larva. Fig. 6. Fourth-stage larva. Fig. 7. Tail of the fourth-stage larva. Fig. 8. Tail of the late fourth-stage larva, male bursa developing.

At 20–22° C. eggs developed to the morula stage (Fig. 2) in 4 days, then passed through a tadpole stage (Fig. 3) and, continuing to elongate, developed to form a recognizable first-stage larva in 9–10 days (Fig. 4). This larva is filled with dark, granular material, and no internal organization is visible. The tail at this stage is already very long, and the larva is quite active within the shell, but hatching does not occur.

The first larval sheath becomes clearly visible at 12–13 days and is quite distinctly loosened from the body at 18 days. As the first and second sheaths are retained at each moult, it is difficult to determine precisely the time when moulting occurs, but this was taken as the time at which the sheath became distinctly separated from the body. The second-stage larva is more sluggish than the first, and during the following 10 days the granular material gradually disappears and the internal organization of the larva becomes visible. After 28–30 days of incubation the body of the larva is transparent, except for the intestine and other organs, and the slightly loosened second larval sheath can be made out. At this stage the second moult was considered to have occurred and the larva was regarded to be in the third or infective stage (Fig. 5).

At this temperature 90–95% of the eggs completed development to the third stage. At lower temperatures development proceeded more slowly, the third stage being reached after 50 days' incubation at 15° C. At higher temperatures development was slightly faster, but an increasing percentage of eggs failed to develop, only 1% development occurring at 36° C.

Hatching did not occur in culture, even when fully developed third-stage larvae were present within the egg membranes, and at 21° C. less than 5% of the larvae hatched over a period of 6 months. However, the larvae immediately became active if liberated by rupture of the membranes, and they also became active and emerged if the membranes were only torn. Both in laboratory culture and under natural conditions hatching required a particular stimulus, but this will be discussed in a later paper.

When the activity of the infective larva has been stimulated, it moves rapidly within the membranes, which rupture, and the larva emerges in a very short time. There appears to be no particular site in the membranes at which rupture occurs, some larvae emerging through the poles, others through the sides of the egg. As the larva emerges the loose first-stage sheath is cast, and usually remains within the egg membranes, but the second-stage sheath is retained. A detailed description of the infective larva of this species has been given in a previous communication (Thomas, 1957).

*Parasitic stages.* Parasitic stages were obtained from the four lambs slaughtered at intervals after dosing with larvae, large numbers, amounting to approximately 25% of the infecting dose (Table 1), being recovered from each lamb.

Fourth-stage larvae were recovered in large numbers from the 5-day-old infection, this being the only stage recovered from this lamb, i.e. the transition from third to fourth stage takes place within the first 5 days after ingestion. These larvae ranged from 1·4 to 4·0 mm. long, and from 0·036 to 0·057 mm. in diameter, the smallest being little larger than third-stage larvae; but in all cases the third

sheath had been cast. The majority of the larvae were within the range 2·4–3 mm. long. At this stage little internal structure could be distinguished, the larvae appearing darkly granular, in marked contrast to the transparent appearance of the third-stage larva. However, in some specimens the oesophagus and intestine could be made out, and the latter was seen to consist of a large number of cells, in contrast to the eight-celled intestine of the infective larva. In the larger specimens a slight thickening and cellular concentration at the posterior end indicated early male differentiation, while the genital primordium had elongated into a sausage-shaped structure. The tail of the larva tapered to a fine point, with a marked notch on the dorsal surface, closely resembling that of the third-stage larva (Figs. 6, 7).

Table 1. *Recovery of* Nematodirus *spp. from experimentally infected lambs*

| Lamb | Dose of larvae | Days after infection | No. recovered | |
|---|---|---|---|---|
| | | | *N. battus* | *N. filicollis* |
| 1 | 50,000 | 5 | 12,000 | 220 |
| 2 | 50,000 | 8 | 9,000 | 400 |
| 3 | 50,000 | 12 | 11,000 | 560 |
| 4 | 50,000 | 15 | 17,000 | 420 |
| 5 | 20,000* | 8 | — | 1,800 |

* *N. filicollis* larvae only.

The lamb killed 8 days after infection also contained large numbers of fourth-stage larvae, differing from those of the 5-day infection only in size and the degree of sex differentiation. The majority of the larvae fell into the range 3–7 mm. in length and 0·039–0·072 mm. in width, the females being rather larger than the males. All the larvae recovered were still in the fourth stage, and in no case had the fourth moult occurred. The larvae were now rather less opaque, and the internal structures could be seen more clearly. In the males a marked thickening was apparent in the region of the bursa, showing some differentiation into the adult bursa structure (Fig. 8) and a dense aggregation of cells in the posterior region of the body indicated the development of the male reproductive system and spicules. In the females the genital rudiment had lengthened and was beginning to differentiate into vagina, ovejectors and oviducts. The cephalic inflation of the cuticle, a characteristic of the adult worms, was becoming apparent at this stage.

In the lamb, slaughtered 12 days after infection, a very high proportion of immature adults was found, the fourth moult having been completed in almost all the specimens examined. These varied from 6 to 15 mm. in length, and from 0·066 to 0·11 mm. in width, the majority being in the range 8–12 mm. In the males the bursa was fully formed and the spicules were present though they were still transparent. In the females the genital system was fully differentiated, but no eggs were present. Thus the fourth moult in this species occurs between the eighth and twelfth day after infection.

The majority of the parasites recovered from the 15-day infection were adults, with approximately 5 % of immature forms and a small proportion of fourth-stage

larvae. A faeces sample from this lamb contained 300 e.p.g. of *N. battus*. Thus the life history of this species is completed in 2 weeks within the host, although maximum egg production would probably not be reached until slightly later.

*Histological examination*

Visual examination of the intestine immediately after slaughter revealed no gross pathological changes in any of the lambs, except for slight patchy congestion at 0–10 ft. in the lamb with a 15-day infection. Examination of fresh portions of intestine mounted in warm saline, under a low-power microscope, showed that in the 5- to 8-day infections few larvae lay entirely exposed on the mucosa, the majority being largely or completely buried in the gut wall, with only a small portion of the body protruding from the surface; and these larvae showed active lashing movements. In the portion from the 12-day infection, and to a greater extent in the 15-day infection, large numbers of immature and mature worms, respectively, were found lying in the mucus layer on the surface of the mucosa, and these showed only sluggish movements.

Study of stained sections of the jejunum confirmed that little pathological change had resulted from the infection. In the case of the lamb with a 5-day infection, numerous larvae were present in cross-section, the majority lying between the villi, at all levels down to the base of the intervillar crypts. Of the portion of intestine taken 5 ft. from the pylorus, seven out of nine sections examined were positive for larvae, at 10 ft. only four out of nine sections were positive, and at 15 ft. no larvae were found in the sections. The diameter of the larvae ranged from 30 to 40$\mu$, suggesting that they were early fourth-stage larvae, and they were identified by comparison with prepared sections of fourth-stage larvae. The presence of the larvae between the villi caused some distortion of the walls, but the epithelial surface appeared normal, except in one case in which rupture of the epithelium and penetration into the lamina propria had occurred, and a second case in which the larva was enclosed within the epithelium.

In the 8-day infection a similar picture was observed, the bulk of the larvae apparently lying in the wall of the first 10 ft. of intestine. The larvae appeared slightly longer, being 45–60$\mu$ in section, and in this case some flattening of the epithelium occurred where it came in contact with the spiny cuticle of the larva, but there was little actual penetration of the epithelium.

The lamb with a 12-day infection showed the most evidence of larval penetration into the intestinal wall, all sections examined at the 5 ft. level containing numerous larvae. In this case the larvae were again larger, being 60–80$\mu$ in section, and probably including a high proportion of immature adults, causing marked distortion of the intervillar spaces. In contrast to the 5- and 8-day infections, there was both flattening and complete erosion of the epithelium in contact with the larvae, which often penetrated into the lamina propria. Penetration below the lamina propria was not observed. At this stage many larvae were also found in the lumen, not in contact with the intestinal wall, indicating some migration back into the lumen. The maximum damage to the intestine at this stage was possibly due to the large

size of the larvae. No evidence of larval activity was found at the 10 or 15 ft. levels from the pylorus.

In the 15-day infection, at 5 ft. from the pylorus, a large proportion of the parasites lay in the lumen of the gut, but not in contact with its wall, and only a small proportion was found in the intervillar spaces. This indicates that the bulk of the infection had migrated back into the lumen as it became adult. At 10 ft. from the pylorus, however, in contrast to the previous results, large numbers of parasites were present, both in the lumen and in the wall of the intestine, the picture resembling that found at the 5 ft. level in the 12-day infection. Even at 15 ft. from the pylorus a small number of parasites were present. Parasites found in the intestinal wall ranged from 60 to 120$\mu$ in section, and thus were largely immature adults. Parasites in the lumen ranged up to 160$\mu$ in section, and probably included both immature and mature adult worms.

In the sections from the 12- and 15-day infections, parasites appeared much more numerous than in the 5- and 8-day infections. This does not reflect any great differences in the worm burdens of the lambs, but is probably mainly due to the fact that the large size of the parasites in the older infections increased the possibility of encountering larvae when a small number of sections was examined. It is difficult to explain the presence of large numbers at 10 ft. from the pylorus in the 15-day infection only. Furthermore, the parasites at this site appeared smaller in section and less advanced than those at the 5 ft. level, and resembled more closely those in the 12-day infection. This could be attributed to delay in development occurring while the larvae were migrating down the intestine to this position. However, the difference in size between the 15-day infection and the rest does not appear sufficient to explain this marked increase in the area invaded.

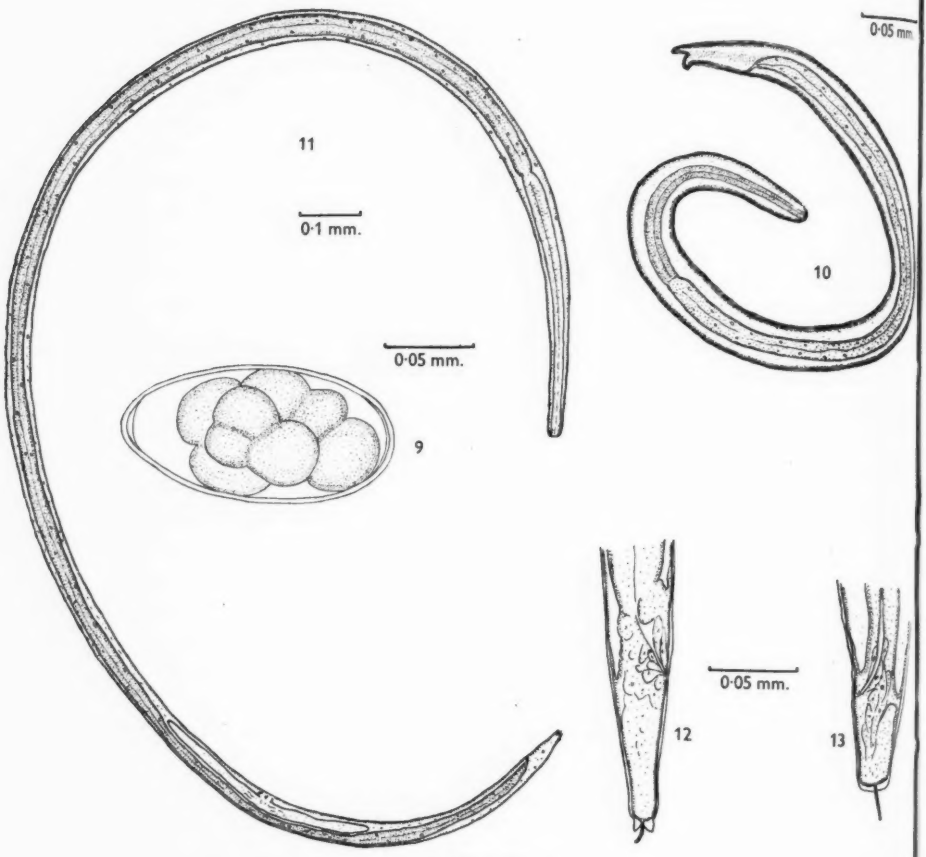
### *Nematodirus filicollis*

*Preparasitic stages.* The egg of *N. filicollis* (Fig. 9) is oval, with one end more rounded than the other, and like other eggs of this genus is relatively large. Mean measurements of 100 eggs taken from the ovejector region of the female worms were $150 \times 75\mu$, the range in length being 134–168$\mu$, and in width 71–78$\mu$. The shell has a uniform thickness of 3$\mu$, and the egg is colourless and transparent, both when it is taken from the female oviduct and when it is recovered from the faeces. At 21° C. eggs pass through the morula stage in 2–3 days and reach the first larval stage in 8–9 days, one day earlier than *N. battus*. The larva is undifferentiated at this stage, and shows considerable activity within the shell, but it does not hatch. At 11–12 days the first-stage sheath is visible, and at 12–16 days it has partly moulted, the larva then becoming a second-stage larva. This is an inactive stage, and during the following 10 days the body becomes transparent, and the internal organization becomes visible. At 24–27 days of incubation development is complete, and the slightly loosened second-stage sheath is visible, this being the third or infective larval stage. Thus the larval stages closely resemble those of *N. battus*, but development proceeds slightly more rapidly.

At higher temperatures development is more rapid, being complete in 20 days at 28° C. and the mortality rate appears to increase more slowly with increase in

temperature than was the case for *N. battus*. At 36° C., however, only 2 % of the eggs incubated developed to the third stage. At temperatures lower than 21° C. development was retarded, being complete in 40–45 days at 15° C., again slightly faster than that of *N. battus*.

The infective larva emerges from the egg in the same way as that of *N. battus* does, the first larval sheath being cast and usually remaining within the shell.




*Nematodirus filicollis*

Figs. 9–13. Fig. 9. Undeveloped egg. Fig. 10. Excheated third-stage–early fourth-stage larva. Fig. 11. Fourth-stage larva. Fig. 12. Tail of the fourth-stage larva. Fig. 13. Tail of the immature adult female.

Emergence of the larva again does not normally occur at incubator temperatures, even up to 36° C. The morphology of the infective larva has already been described and its differentiation from that of *N. battus* and *N. spathiger* discussed (Thomas, 1957).

Parasitic stages of *N. filicollis* were recovered from the four lambs infected with

mixed larvae, only 5% *N. fillicollis* larvae being present in the infecting dose, resulting in burdens of about 500 *N. filicollis* in the lambs.

Fourth-stage larvae were recovered from both the 5-day and 8-day infections, the larvae in the former being largely in the range 1·3–2·5 mm. in length, and in the latter 2·0–4·1 mm. The smallest larvae recovered from the 5-day infection measured only 600$\mu$ in length and possessed a short forked tail with no digitate process (Fig. 10); these were possibly exsheathed third-stage larvae. Larvae of 1 mm. or more in length had a forked tail with a prominent digitate process 8–10$\mu$ long, and it would therefore appear that the digitate process develops in the transition from third to fourth stage. The fourth-stage larva resembled that of *N. battus*, except in the character of the tail. In both 5-day and 8-day infections the larvae were much smaller than those of *N. battus* (Figs. 11–12).

From the 12-day infection mostly fourth-stage larvae were again recovered. They ranged from 3·5 to 6·5 mm. in length and, at the upper limit of this range, a small proportion of early immature adults were present. The immature adult female may be distinguished from the fourth-stage larva by the shape of the tail, which tends to be flattened, and not deeply notched as it is in the larva (Fig. 13). At the comparable stage the *N. battus* burden was composed largely of immature adults of 6–15 mm. long. The transition from the fourth-stage larva to the immature adult appears to occur at approximately the same stage of growth in both species, i.e. at about 6 mm. in length, but the rate of development of *N. filicollis* is rather slower than that of *N. battus*.

The lamb slaughtered 15 days after infection contained mainly immature worms, together with approximately 25% mature adults and a small proportion of fourth-stage larvae. By comparison, the 15-day *N. battus* infection had largely reached the mature adult stage, and it is apparent that, whereas the *N. battus* burden became adult by the end of the second week after infection, the *N. filicollis* burden would only become adult during the third week.

*Histological examination*

One lamb was infected with 20,000 *N. filicollis* larvae, and was killed 8 days after infection. Visual examination of the intestine showed no pathological changes, as would be expected from such a small dose of larvae. Histological examination showed the presence of larvae, both in contact with the wall of the intestine, and lying in the intervillar crypts, the picture closely resembling that seen in the 5–8-day *N. battus* infections. In view of the close similarity of the life cycles of the two species, it is therefore probable that *N. filicollis* larvae follow the same sequence of migration into and out of the wall of the intestine as those of *N. battus*.

## DISCUSSION

The results show that the life histories of *N. battus* and *N. filicollis* follow the general pattern for the genus, and are broadly similar to those of *N. spathiger* (Kates & Turner, 1955) and *N. helvetianus* (Herlich, 1954), the latter being a parasite of cattle.

The preparasitic stages of these four species are similar in appearance, and the developmental times are of the same order, all being relatively long compared with those of other common gastro-intestinal nematodes. There are, however, slight but consistent differences in the rate of development which are probably inherent and not due to minor variations in culture technique, as was suggested by Kates & Turner (1955). Preparasitic development at 21° C. is apparently complete in 17–20 days in *N. helvetianus* and 18–22 days in *N. spathiger*, as compared with 24–27 days for *N. filicollis* and 28–30 days for *N. battus*.

There appears to be a marked difference in the ease with which hatching occurs in the laboratory between *N. spathiger* and *N. helvetianus* on the one hand, and *N. battus* and *N. filicollis* on the other. The former species hatch readily at temperatures from 20 to 28° C., whereas the latter show a very low percentage hatch at these temperatures and appear to require certain specific environmental conditions before hatching will occur. A detailed investigation of the hatching stimulus and its relation to the epizootiology of *Nematodirus* disease will be the subject of a further paper.

Slight differences also occur in the rate of parasitic development in these four species. At 15 days after infection the *N. battus* burden was largely mature, whereas that of *N. filicollis* was largely immature, with a small proportion of mature adults present. At the same period after infection *N. spathiger* was also reported to be mostly immature with a small proportion of mature forms, whereas *N. helvetianus* was completely immature. Thus parasitic development is completed most rapidly in *N. battus*, the prepatent periods being 2 weeks for *N. battus*, 2–3 weeks for *N. filicollis* and *N. spathiger*, and 3 weeks for *N. helvetianus*. Herlich (1954) and Kates & Turner (1955) describe a stage termed the fifth-stage larva, which corresponds to the immature adult form, but the use of this term implies a further moult between this fifth-stage larva and the adult, which does not occur, and it was, therefore, avoided in the present work. Kates & Turner (1955) suggested that the difference in prepatent period between *N. spathiger* and *N. helvetianus* might be due either to intrinsic species differences or to the influence of the different hosts involved. The results discussed above show that similar differences in prepatent period occur between species in the same host, and it must, therefore, be concluded that these are intrinsic species variations.

The parasitic development of *N. battus* and *N. filicollis* involves a migration of larvae into the depths of the villi, and the subsequent return of the maturing parasites to the lumen, thus following the pattern described for *N. spathiger* and *N. helvetianus*. The fact that in the 5- and 8-day infections very few larvae were observed lying in the lumen of the intestine, and the uniformity with which the phenomenon occurs in all four species, indicate that this migration is an integral part of the life cycle. This migratory behaviour is, in fact, common to most of the gastro-intestinal nematodes of ruminants, except when a blood migration occurs. There is, however, some variation in the degree of migration occurring, and *Nematodirus* species appear to fall between the two extremes represented by species such as *Cooperia curticei*, in which the larvae migrate into the depths of the intestinal crypts but never penetrate the mucosa (Andrews, 1939), and *Oesopha-*

*gostomum columbianum* in which deep penetration of the intestinal wall is very common (Fourie, 1936).

Kates & Turner (1955) showed that when heavy infecting doses of *N. spathiger* larvae were used, the migration of the developing larvae caused extensive damage to the wall of the intestine. These authors (1953) also showed that, in experimental infections with *N. spathiger*, the clinical effects, namely, diarrhoea and loss of weight, appeared during the second week after infection. Similarly, Herlich & Porter (1953) found that diarrhoea began in calves 15 days after infection with *N. helvetianus* larvae. It was concluded, therefore, that the pathogenic effects of these infections were due to the tissue damage caused by the larval stages during the period of intimate contact with the intestinal wall.

No symptoms of disease were produced by the artificial infections used in the present work, probably because small numbers of larvae were used. However, the clinical picture in *Nematodirus* disease in Britain is characterized by profuse diarrhoea, with marked dehydration and loss of condition, so that it closely resembles that caused by *N. spathiger* and *N. helvetianus* Kingsbury (1953) Thomas & Stevens (1956). In view, therefore, of the similarity of both the clinical picture in natural infections, and the developmental stages of these four species, it is reasonable to assume that *Nematodirus* disease in Britain, as in the U.S.A., is due to damage to the intestinal wall caused, towards the end of the prepatent period, by the developing parasites.

## SUMMARY

1. The life history of *N. battus* is described, and a comparative description of the life history of *N. filicollis* is given.

2. The life histories of these two species are compared with those of *N. spathiger* and *N. helvetianus*, two closely related species, and are shown to follow the same basic pattern, with minor variations in timing which appear to be specific in nature, and not related to differences in culture methods or host species.

3. The pathogenesis of *Nematodirus* species is discussed and related to the migration of larvae into the intestinal mucosa during development.

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# FIELD STUDIES ON THE SEASONAL INCIDENCE OF *NEMATODIRUS BATTUS* AND *N. FILICOLLIS* IN SHEEP

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(With 4 Figures in the Text)

## INTRODUCTION

The genus *Nematodirus* was formerly considered to be relatively non-pathogenic to sheep, and of little importance in Britain. However, in recent years a serious parasitic disease has been reported in young lambs, associated with heavy infestations of *N. battus* and *N. filicollis*. In America, Kates & Turner (1953) reviewed the literature on the pathogenicity of the genus and reproduced the disease by experimental infection with *N. spathiger*. The condition is characterized by its acute nature, the suddenness with which it appears, and the fact that the disease tends to be restricted to the young lamb flock in late spring–early summer. Thus the picture presented by *Nematodirus* disease differs markedly from that typical of parasitic gastro-enteritis, and the differences suggest that the biology of this group differs considerably from that of other common gastro-intestinal parasites.

Life history studies (Boulenger, 1915; Kates & Turner, 1955; Thomas, 1959) have shown that the preparasitic stages of this genus develop within the egg, the infective larva being the only free-living stage, and the various stages of development have been described. However, field studies on these parasites have been limited mainly to the period when disease outbreaks occur. Kingsbury (1953), and Thomas & Stevens (1956), stated that the disease season in north-east England extended over the period May–June, and in a preliminary report on pasture sampling for infective larvae the latter authors showed that larval activity was at its height in late April–early June. Baxter (1957) found that in Northern Ireland outbreaks occurred from late April until early July.

The present investigation was undertaken to provide detailed information on the incidence of *N. battus* and *N. filicollis* throughout the year. Observations were made over a 3-year period on the variations in the *Nematodirus* egg output of sheep, the fluctuations in the *Nematodirus* populations in sheep, and the availability of the infective larvae on pasture. The work was carried out on the College farm at Nafferton, Northumberland.

## 1. THE SEASONAL FLUCTUATIONS IN *NEMATODIRUS* EGG OUTPUT OF SHEEP

### *Material and methods*

A small flock of twenty halfbred (Border Leicester × Cheviot) ewes with twin lambs was put on a permanent pasture of 30 acres on 1 May 1954. They remained

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on this pasture, except for a short period in June–July, when the lambs were weaned. In November 1954, the ewes and lambs were transferred to another permanent pasture of 24 acres. The flock was then kept on this pasture during 1955–57, except for short periods at lambing and weaning, when the ewes rejoined the main farm flock. Approximately half the lambs were slaughtered each year, while the remainder were kept on as hoggs and slaughtered the following year. Thus each spring during 1955–57 the same pasture carried twenty ewes with twin lambs together with approximately twenty hoggs. (To avoid confusion the term 'lamb' is used for animals up to 1 year old, and 'hogg' for animals between 1 and 2 years old.) All the sheep were ear-tagged for identification purposes.

Faeces samples were taken from the lambs and hoggs at weekly intervals, while the ewes were sampled weekly in 1954–55, and fortnightly in 1956–57. Each sample was taken from the rectum and placed in a jar numbered for identification. Samples were not collected from the ewes during the period just before and just after lambing, and sampling of the lambs began at approximately 6 weeks old. Egg counts were carried out by the McMaster technique (Gordon & Whitlock, 1939), using 2 g. faeces samples, and counting six slide chambers for each sample. From 31 May 1955, differential counts of *N. battus* and *N. filicollis* eggs were made.

## *Results*

The egg counts are shown graphically in Figs. 1–3.

### Nematodirus *egg counts in ewes*

1954. Very low *Nematodirus* counts were recorded in the ewes from 25 May, when sampling began, until 15 June. An average count of 10–20 eggs per g. (e.p.g.) was recorded for the flock, representing counts of 50–100 e.p.g. for individual ewes, some ewes showing no eggs throughout. No eggs were detected from the whole flock after 15 June, until sampling ceased on 11 January 1955.

1955. Sampling began on 26 April, and from this date only sporadic counts were recorded from eight individual ewes, between May and December, the remainder showing no *Nematodirus* eggs throughout until sampling ceased on 10 January 1956.

1956. Sampling began on 20 March. Eggs of both *N. battus* and *N. filicollis* were first recorded from three ewes on 1 May, and from seven ewes on 8 May. Small numbers of eggs continued to be recorded from individual animals until 12 June, and from a single ewe in October. The counts recorded were again of the order of 50–100 e.p.g.

1957. Sampling began on 2 April, and *N. battus* eggs were recorded from five ewes from 9 April until 4 June. *N. filicollis* eggs were detected on only one occasion, from a single ewe on 7 May.

Thus with the exception of 1955, when no definite trend could be established, *Nematodirus* counts occurred mainly in the period April–June, although the counts were extremely low throughout, and a considerable percentage of the ewes showed no eggs.

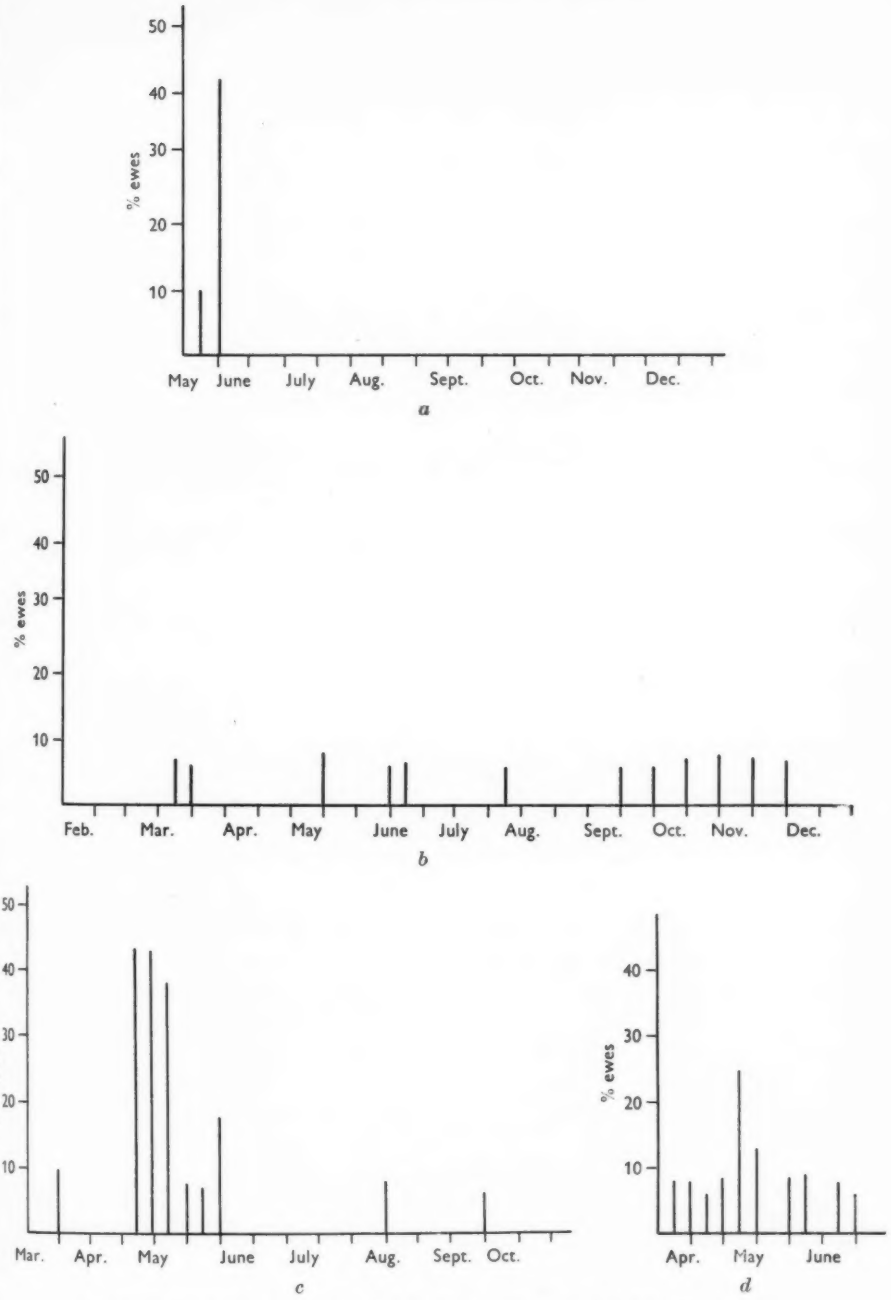



Fig. 1. Percentage of ewes passing *Nematodirus* eggs. Fig. 1*a*. Ewes. 1954. Fig. 1*b*. Ewes. 1955. Fig. 1*c*. Ewes. 1956. Fig. 1*d*. Ewes. 1957.

Nematodirus *egg counts in hoggs*

1955. The lambs from the 1954 flock were carried on as hoggs in 1955, and egg counts were recorded weekly until the last pair was slaughtered in December. No eggs were recorded from 4 January until 22 March, when counts occurred in

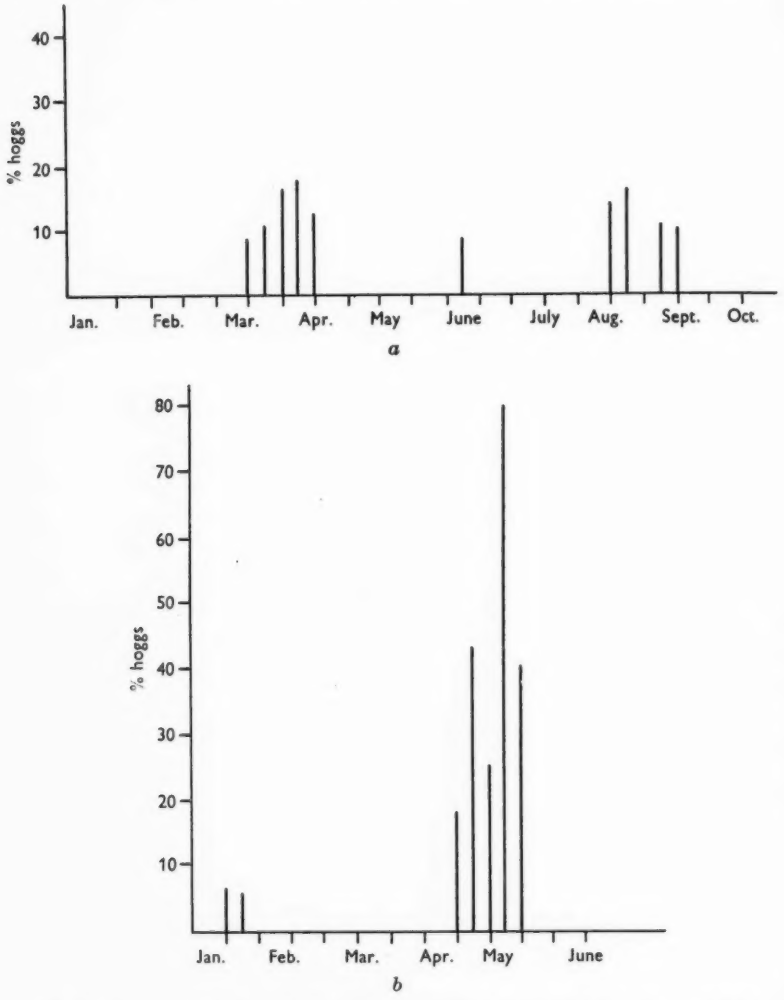



Fig. 2. Percentage of hoggs passing *Nematodirus* eggs. Fig. 2*a*. Hoggs. 1955. Fig. 2*b*. Hoggs. 1956. Fig. 2*c*. Hoggs. 1957.

three animals. Small numbers of eggs continued to be detected until 19 April, the mean flock count reaching 24 e.p.g., and the maximum individual count being 250 e.p.g. Further counts occurred sporadically in individual animals in June, August and September.

1956. Twenty-three lambs from the 1955 flock were kept as hoggs in 1956 and slaughtered in groups of three from 2 March until 5 June. Egg counts were recorded weekly during this period. No eggs were found from 24 January until 24 April, when two counts of 30 e.p.g. *N. battus* and 30 e.p.g. *N. filicollis* were recorded from a total of eleven hoggs. Low counts of 30–200 e.p.g. continued in individual animals until 29 May, when the remaining four hoggs showed no eggs.

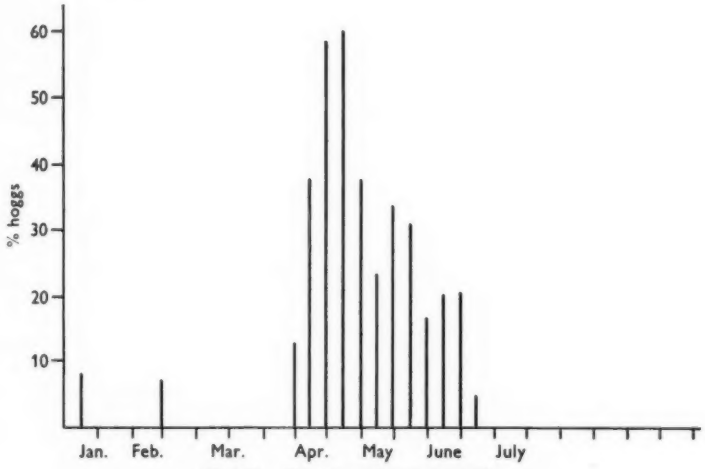



Fig. 2c. For legend see opposite.

1957. Eighteen lambs from the 1956 flock were run as hoggs in 1957 and sampled weekly. Single *N. filicollis* counts occurred in January and February. *N. battus* eggs were first recorded on 16 April, and both species continued to be detected in individual animals through April, May and June. Mean maxima of 56 e.p.g. *N. battus* and 18 e.p.g. *N. filicollis* were recorded for the flock, maximum individual counts being 120 e.p.g. for both species.

The egg-count pattern in the hoggs therefore resembled that in the ewes, very low numbers being recorded throughout, and these occurring mainly in the April–May period. In view of the small number of eggs recorded, no conclusions could be drawn regarding the relative incidence of the two species.

### Nematodirus *egg counts in lambs*

1954. Sampling began on 25 May when high egg counts were already present, and the mean egg count for the flock reached a peak a week later on 1 June. The count then fell rapidly over the next 4 weeks to reach a low level of 220 e.p.g. on 29 June. A slower fall continued until 30 November, after which no eggs were detected.

1955. The mean flock egg count rose rapidly from a very low level on 26 April to reach a maximum of 1800 e.p.g. on 24 May. The count then fell rapidly again over the next 4 weeks, dropping to 50 e.p.g. on 21 June, and then fluctuated at a low level until 24 January 1956, when the last eggs were recorded. Differential

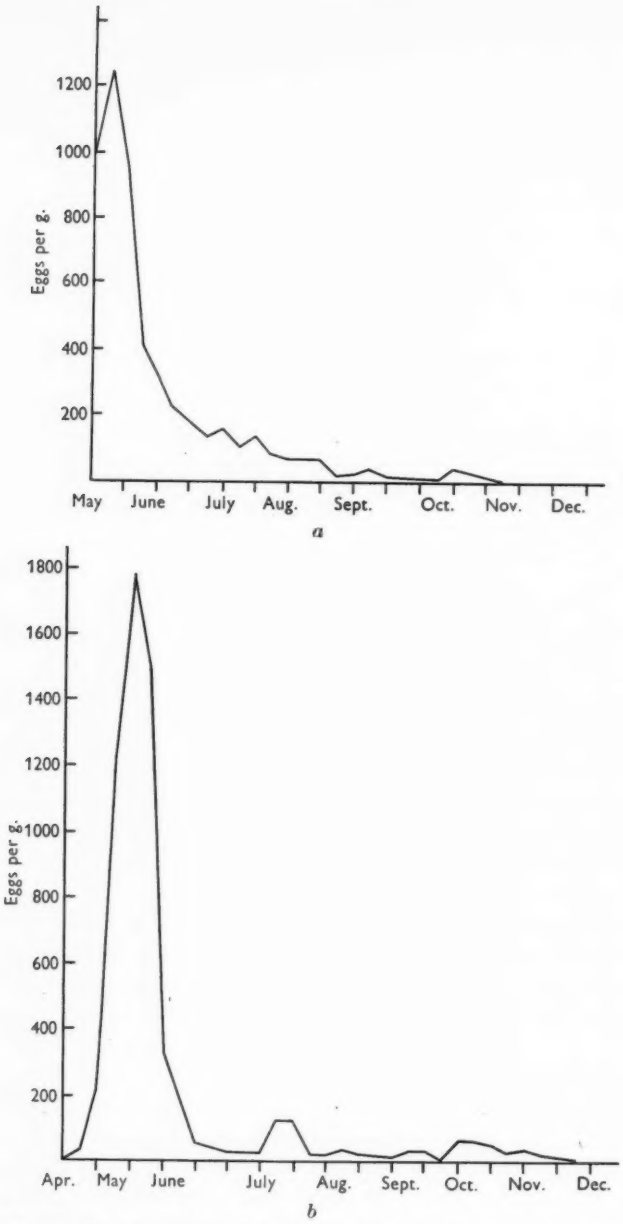
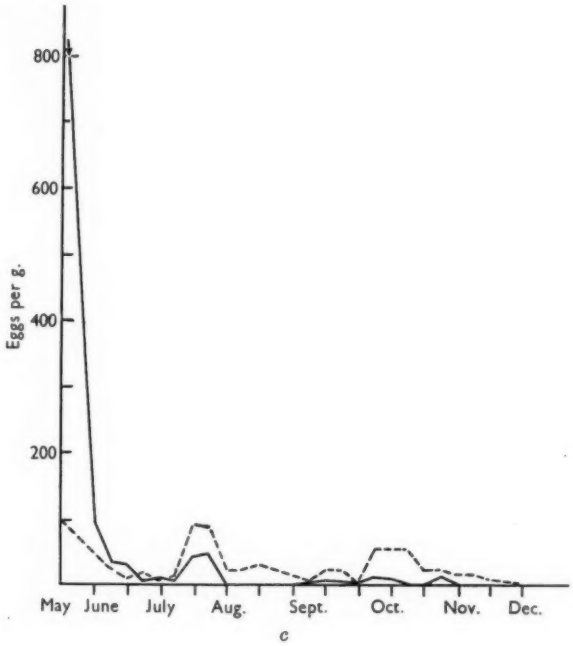
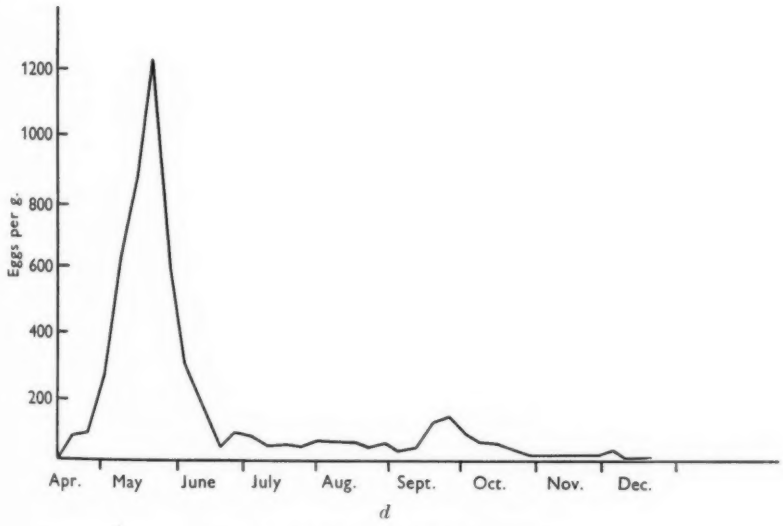



Fig. 3. Mean *Nematodirus* egg counts in lambs. Fig. 3*a*. Lambs. 1954. Total *Nematodirus* egg count. Fig. 3*b*. Lambs. 1955. Total *Nematodirus* egg count. Fig. 3*c*. Lambs. 1955. *Nematoridus* egg counts. ——, *N. battus*; – – –, *N. filicollis*. Fig. 3*d*. Lambs. 1956. Total *Nematoridus* egg count. Fig. 3*e*. Lambs. 1956. *Nematodirus* egg counts. ——, *N. battus*; – – –, *N. filicollis*. Fig. 3*f*. Lambs. 1957. Total *Nematodirus* egg count. Fig. 3*g*. Lambs. 1957. *Nematodirus* egg counts. ——, *N. battus*; – – –, *N. filicollis*.

counts of *N. battus* and *N. filicollis* were begun on 31 May and showed that at this time eggs of the two species were present in the proportions of 94% *N. battus* and 6% *N. filicollis*, the respective counts being 1300 e.p.g. and 100 e.p.g. The *N. filicollis* egg count, however, declined less markedly than that of *N. battus*, and

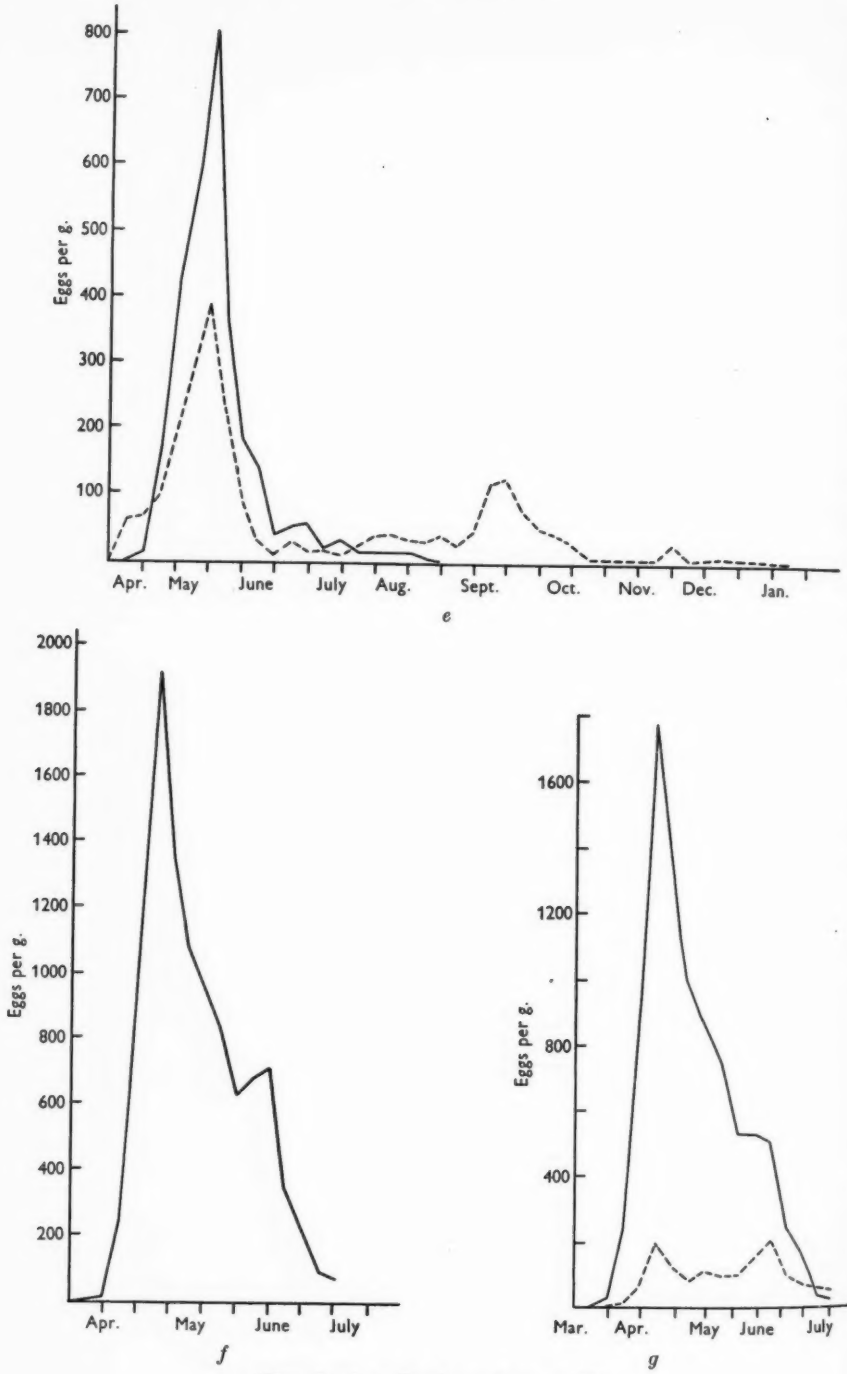



*c*




*d*

Fig. 3*c*, *d*. For legend see opposite.




**Figs. 3*e*, *f*, *g*.** For legends see p. 392.

persisted until 24 January, whereas the last *N. battus* eggs were recorded on 15 November.

1956. The mean flock egg count again rose rapidly from a low level on 10 April to a maximum of 1200 e.p.g. on 22 May, and then fell to a low level over the following four weeks, dropping to 40 e.p.g. on 19 June. Differential counts for *N. battus* and *N. filicollis* showed that both species followed the same pattern of increase and decrease in egg count, the proportions at the time of maximum count being 66% *N. battus* and 34% *N. filicollis*. The decline in egg count of *N. filicollis* was, however, again slower than that of *N. battus*, and eggs were recorded until 22 January 1957, whereas the last *N. battus* eggs were recorded on 28 August. In addition there was a slight rise in the *N. filicollis* egg count in September.

1957. The general pattern of egg output resembled that of previous years. The mean flock egg count rose from a very low level on 19 April, to reach a maximum of 2000 e.p.g. on 30 April, some 3–4 weeks earlier than in 1954–56, and again fell rapidly to reach a low level of 100 e.p.g. on 9 June. Differential counts showed a similar pattern for both *N. battus* and *N. filicollis*, being less clear-cut in the case of *N. filicollis*, because of the low maximum count recorded. At the peak of egg production the proportions were 90% *N. battus* and 10% *N. filicollis*. This corresponds closely with the results of 1955, but both the proportional and absolute counts of *N. filicollis* were considerably lower than in 1956.

## 2. SEASONAL FLUCTUATIONS IN *NEMATODIRUS* POPULATIONS, AS INDICATED BY POST-MORTEM EXAMINATION

### *Material and methods*

No lambs were slaughtered in 1954, the lambs being retained as hoggs and slaughtered in groups of four at approximately monthly intervals throughout 1955. Of the 1955 lamb crop, two lambs were slaughtered on 24 May, 10 June and 23 June, and four on 15 July and 11 August. These results were augmented by slaughtering lambs from the main farm flock, running on similar pastures, between 2 May and 17 June, as indicated in Table 3. The remainder of the 1955 lamb crop were carried on as hoggs, and these were slaughtered in groups of three, at fortnightly intervals from 2 March until 8 June 1956, in order to give a more detailed picture of the changes in the worm population over this critical period. Of the 1956 lamb crop, a pair of lambs was slaughtered each week from 20 April until 19 July. The remainder were carried on as hoggs through 1957. No post-mortem examinations were carried out in 1957.

Slaughtering was carried out at the Hexham abbattoir of the Fatstock Marketing Corporation. The intestine was tied off, removed and returned to the laboratory, where it was opened along its length and its contents were washed into a 100-mesh sieve. The material was well washed to remove colouring matter and debris, and then stored in 10% formalin, and examined later for *Nematodirus* and other species, using a dilution technique. In the case of lambs slaughtered in 1956, the intestine was dealt with in 5–10 ft. sections to analyse the distribution of the

worm population, and the material was washed through 100- and 300-mesh sieves to retain larval stages as well as adults. Examination for larval stages was carried out under a dissecting microscope, using a dilution technique.

*Results*

*Hoggs.* Four to six hoggs were slaughtered at approximately monthly intervals during 1955, and the numbers of *Nematodirus* worms recovered at post-mortem are given in Table 1. Total *Nematodirus* burdens were low throughout the year except during March and April when two counts of 4800 and 1600 worms were recorded. Outside this period only three hoggs were found to contain *Nematodirus*, harbouring six, one and six worms, respectively—numbers which are of little

Table 1. *Hoggs* 1955. *Post-mortem worm counts*

| Date of slaughter | Hogg no. | *Nematodirus* recovered | Date of slaughter | Hogg no. | *Nematodirus* recovered |
|---|---|---|---|---|---|
| 5 Jan. | 1 | Nil | 5 May | 22 | Nil |
| | 2 | Nil | 29 June | 23 | Nil |
| | 3 | Nil | | 24 | Nil |
| | 4 | 6 | | 25 | Nil |
| 12 Feb. | 5 | Nil | | 26 | Nil |
| | 6 | Nil | 28 July | 27 | Nil |
| | 7 | Nil | | 28 | Nil |
| | 8 | 1 | | 29 | Nil |
| | 9 | Nil | | 30 | Nil |
| | 10 | Nil | 2 Sept. | 31 | Nil |
| 21 Mar. | 11 | 4800 | | 32 | Nil |
| | 12 | 270 | | 33 | Nil |
| | 13 | 10 | | 34 | Nil |
| | 14 | Nil | 28 Oct. | 35 | Nil |
| | 15 | Nil | | 36 | Nil |
| | 16 | Nil | | 37 | Nil |
| 26 Apr. | 17 | Nil | | 38 | Nil |
| | 18 | Nil | 14 Dec. | 39 | Nil |
| | 19 | 10 | | 40 | Nil |
| | 20 | 1600 | | 41 | Nil |
| | 21 | Nil | | 42 | Nil |

significance. During March three out of six hoggs, and in April two out of five hoggs, contained *Nematodirus*. Thus the worm burden pattern and the egg count pattern are complementary, both indicating a very low percentage infestation in the hoggs, except during March–April. Of the total *Nematodirus* recovered, over 99% were *N. filicollis*, and only four *N. battus* adults were identified.

Of the hoggs slaughtered during the period March–June 1956, only four contained adult *Nematodirus* at post-mortem, and these contained only very small numbers, the highest number recorded being 110. However, larval counts were made on these hoggs, and twelve out of twenty-three contained fourth-stage larvae, the counts ranging up to 2000 and consisting almost entirely of *N. filicollis*, with very few *N. battus* larvae present (Table 2).

*Lambs.* Groups of lambs from the experimental flock and the main farm flock

were slaughtered at approximately fortnightly intervals from May to August 1955. The *Nematodirus* burden increased markedly from 2 May until 24 May, and then declined steadily until a low average figure was reached in August. The mean proportions of the two species present were 88% *N. battus* and 12% *N. filicollis* (Table 3).

Table 2. *Hoggs* 1956. *Post-mortem worm counts*

| | | *Nematodirus* recovered | | | |
|---|---|---|---|---|---|
| | | *N. filicollis* | | *N. battus* | |
| Date of slaughter | Hogg no. | Adults | Larvae | Adults | Larvae |
| 2 Mar. | 1 | — | — | 12 | — |
| | 2 | 110 | 840 | — | — |
| | 3 | — | 2050 | — | — |
| 16 Mar. | 4 | — | — | — | — |
| | 5 | — | — | — | — |
| | 6 | — | 1650 | 3 | 30 |
| 29 Mar. | 7 | — | — | — | — |
| | 8 | — | — | — | — |
| | 9 | — | — | — | — |
| 13 Apr. | 10 | — | — | — | — |
| | 11 | — | — | — | 5 |
| | 12 | — | — | — | — |
| 27 Apr. | 13 | — | 5 | — | — |
| | 14 | — | 40 | — | — |
| | 15 | — | — | 5 | — |
| 11 May | 16 | — | 1160 | — | — |
| | 17 | — | 290 | — | — |
| | 18 | — | 160 | — | 10 |
| 25 May | 19 | — | — | — | — |
| | 20 | — | — | — | — |
| | 21 | — | 190 | 1 | — |
| 8 June | 22 | — | 18 | — | — |
| | 23 | — | 740 | — | — |

In 1956 a pair of lambs from the experimental flock was slaughtered each week from 20 April until 8 July, and detailed post-mortem counts were made (Table 4). As in 1955 the *Nematodirus* burden rose rapidly in May and reached a peak at the beginning of June. After 1 June the burden declined, but the fall was less marked than in 1955 and an appreciable burden was still present on 10 July, when slaughtering ceased.

The distribution of *N. battus* and *N. filicollis* in the small intestine is shown in Table 5, which is based on the post-mortem examination of twenty lambs. The table shows that all stages of both species are almost entirely confined to the first 20–25 ft. of the small intestine, reaching a peak at 10–15 ft. in the case of *N. battus* adults, and at 5–10 ft. in the case of *N. filicollis*.

### 3. SEASONAL VARIATIONS IN NUMBERS OF AVAILABLE *NEMATODIRUS* LARVAE ON PASTURE

The infective larva is the only free-living stage in the *Nematodirus* life history, and it may be easily distinguished from those of other species by the long sheath tail. The larvae of *N. battus* and *N. filicollis* can also be differentiated from each other without difficulty (Thomas, 1957). Preliminary observations showed that the period of high faecal egg counts was restricted to the period May–June, and that,

Table 3. *Lambs* 1955. *Post-mortem worm counts*

| Date of slaughter | | Lamb no. | *Nematodirus* recovered |
|---|---|---|---|
| 2 May | Farm flock | 1 | 225 |
| | | 2 | 15 |
| | | 3 | 3,200 |
| | | 4 | 2,700 |
| | | 5 | 1,325 |
| 24 May | Farm flock | 6 | 4,000 |
| | | 7 | 4,150 |
| | | 8 | 2,410 |
| | | 9 | 40,600 |
| | | 10 | 74,000 |
| 10 June | Expt. flock | 11 | 105 |
| | | 12 | 10,900 |
| 17 June | Farm flock | 13 | 1,300 |
| | | 14 | 6,400 |
| | | 15 | 510 |
| | | 16 | 2,750 |
| | | 17 | 1,000 |
| | | 18 | 85 |
| 23 June | Expt. flock | 19 | 10 |
| | | 20 | 2,960 |
| 15 July | Expt. flock | 21 | 310 |
| | | 22 | 140 |
| | | 23 | 40 |
| | | 24 | 3,100 |
| 11 Aug. | Expt. flock | 25 | 0 |
| | | 26 | 150 |
| | | 27 | 165 |
| | | 28 | 600 |

outside this period, very low numbers of eggs were passed. Assuming a reasonably uniform rate of development of these eggs it could be expected that the infective larvae of these species would also appear over a relatively restricted period. Furthermore, the rapidity with which the *Nematodirus* burden builds up in lambs in the spring suggests that infective larvae are available in large numbers at this time. It was therefore decided to investigate the fluctuations in *Nematodirus* larval populations on a normal pasture.

### *Material and methods*

The pastures selected were those which had carried the experimental flock in 1954 and 1955–57, in order that data from the flock and from pasture sampling could be correlated. Sampling of the pastures was carried out at fortnightly intervals over the years 1955–57, except at times of high larval activity, when weekly samples were taken.

The method used was that of Michel & Parfitt (1955), modified to reduce the amount of time and handling involved without loss of efficiency. The grass samples

Table 4. *Lambs* 1956. *Post-mortem worm counts*

| Date of slaughter | Lamb no. | Total *Nematodirus* adults recovered | Species distribution | | | |
|---|---|---|---|---|---|---|
| | | | *N. filicollis* | | *N. battus* | |
| | | | Adults | Larvae | Adults | Larvae |
| 20 Apr. | 1 | 231 | 110 | 168 | 121 | 580 |
| | 2 | 21 | 13 | 8 | 8 | 8 |
| 27 Apr. | 3 | 246 | 58 | 94 | 188 | 193 |
| | 4 | 661 | 65 | 126 | 596 | 583 |
| 4 May | 5 | 364 | 77 | 8 | 287 | 47 |
| | 6 | 1,414 | 65 | 145 | 1,349 | 499 |
| 11 May | 7 | 1,838 | 951 | 4 | 887 | 38 |
| | 8 | 5,533 | 1,267 | 50 | 4,266 | 221 |
| 18 May | 9 | 768 | 200 | 42 | 568 | 52 |
| | 10 | 3,852 | 1,334 | 19 | 2,518 | 56 |
| 25 May | 11 | 5,736 | 2,205 | 167 | 3,531 | 907 |
| | 12 | 5,066 | 2,337 | 50 | 2,729 | 46 |
| 1 June | 13 | 10,426 | 2,498 | 200 | 7,928 | 342 |
| | 14 | 4,844 | 882 | 59 | 3,962 | 149 |
| 8 June | 15 | 3,564 | 553 | 910 | 3,011 | 483 |
| | 16 | 3,403 | 1,875 | 135 | 1,528 | 303 |
| 15 June | 17 | 1,720 | 1,190 | 15 | 530 | 39 |
| | 18 | 3,111 | 554 | 31 | 2,557 | 42 |
| 22 June | 19 | 3,272 | 876 | 138 | 2,396 | 454 |
| | 20 | 12,561 | 3,793 | 308 | 8,768 | 877 |
| 10 July | 21 | 937 | 250 | 64 | 687 | 3 |
| | 22 | 2,279 | 548 | 240 | 1,731 | 6 |

were plucked instead of being cut with scissors, because according to Crofton (1954*b*) this gives more consistent results. The grass was soaked in water in a bucket for 24 hr., then removed, squeezed out and resoaked. The contents of the first bucket were then poured through two sieves of 100 and 400 mesh, respectively, and well washed with a fine jet of water. The contents of the 100-mesh sieve were discarded. The contents of the 400-mesh sieve were then pipetted into centrifuge tubes and centrifuged, first in water and then in salt solution, to concentrate the larvae by flotation. This was repeated for the second soaking and the total number of larvae recovered was recorded.

Examination of the washings passing through the 400-mesh sieve, and of the debris in the 100-mesh sieve, showed that only 6 % of the larvae were lost in these two ways. A further loss occurred in the collection of the material in the 400-mesh sieve, but this could not be estimated. This method shows a considerable saving in time as the material is handled only once in sieving, whereas several operations are involved in the original sedimentation technique. The method was checked by using grass to which a known number of larvae had been added, a final recovery of 53 % being obtained, as compared with 43 % reported by Michel & Parfitt. Analysis of a number of samples showed that 88 % of the total number of larvae recovered were obtained from the first soaking of the grass, and 12 % from the second soaking. Results were expressed as the number of larvae recovered per pound of herbage.

Table 5. *Lambs* 1956. *Distribution of* Nematodirus

| Stage of development | % distribution in small intestine | | | | | |
|---|---|---|---|---|---|---|
| | 0–5 ft. | 5–10 ft. | 10–15 ft. | 15–20 ft. | 20–25 ft. | Over 25 ft. |
| | | | *Nematodirus battus* | | | |
| Adults | 13 | 24 | 38 | 21 | 2 | 2 |
| Immature adults | 22 | 41 | 30 | 6 | 1 | — |
| Larvae | 42 | 34 | 16 | 5 | 2 | 1 |
| | | | *Nematodirus filicollis* | | | |
| Adults | 20 | 45 | 22 | 12 | 1 | — |
| Immature adults | 32 | 46 | 14 | 8 | — | — |
| Larvae | 23 | 22 | 26 | 19 | 8 | 2 |

*Results* (*Fig.* 4)

The pasture sampled in 1955 had carried the experimental flock in April–June 1954, and after this date the pasture carried only a few adult sheep during the following 12 months. Sampling began on 15 March 1955 and continued until 13 October. The first positive sample recorded on 31 March showed two *N. filicollis* larvae per pound of herbage. Low counts of *N. filicollis* larvae were recorded in April; then, at the beginning of May, the count rose very rapidly to 127 larvae per pound, of which 90 % were *N. battus* larvae. A peak of 170 larvae per pound was reached on 9 May, after which the count fell equally rapidly to reach a low level by the end of June. Very low counts were recorded during August and September, and sampling ceased in October, when no larvae were recovered. These results were reported briefly in an earlier paper (Thomas & Stevens, 1956).

1956–57. The pasture sampled was that to which the experimental flock was moved in November 1954, and which carried this flock throughout 1955–57. Sampling began on 2 March 1956, when a count of seven *N. battus* and seventeen *N. filicollis* larvae per pound was recorded. The count rose rapidly to a peak of 158 larvae per pound on 24 April, fell to a low level in early May, then rose again to a second peak of 176 larvae per pound on 24 June, falling to a low level once more by mid-July. Both *N. battus* and *N. filicollis* followed the same pattern and

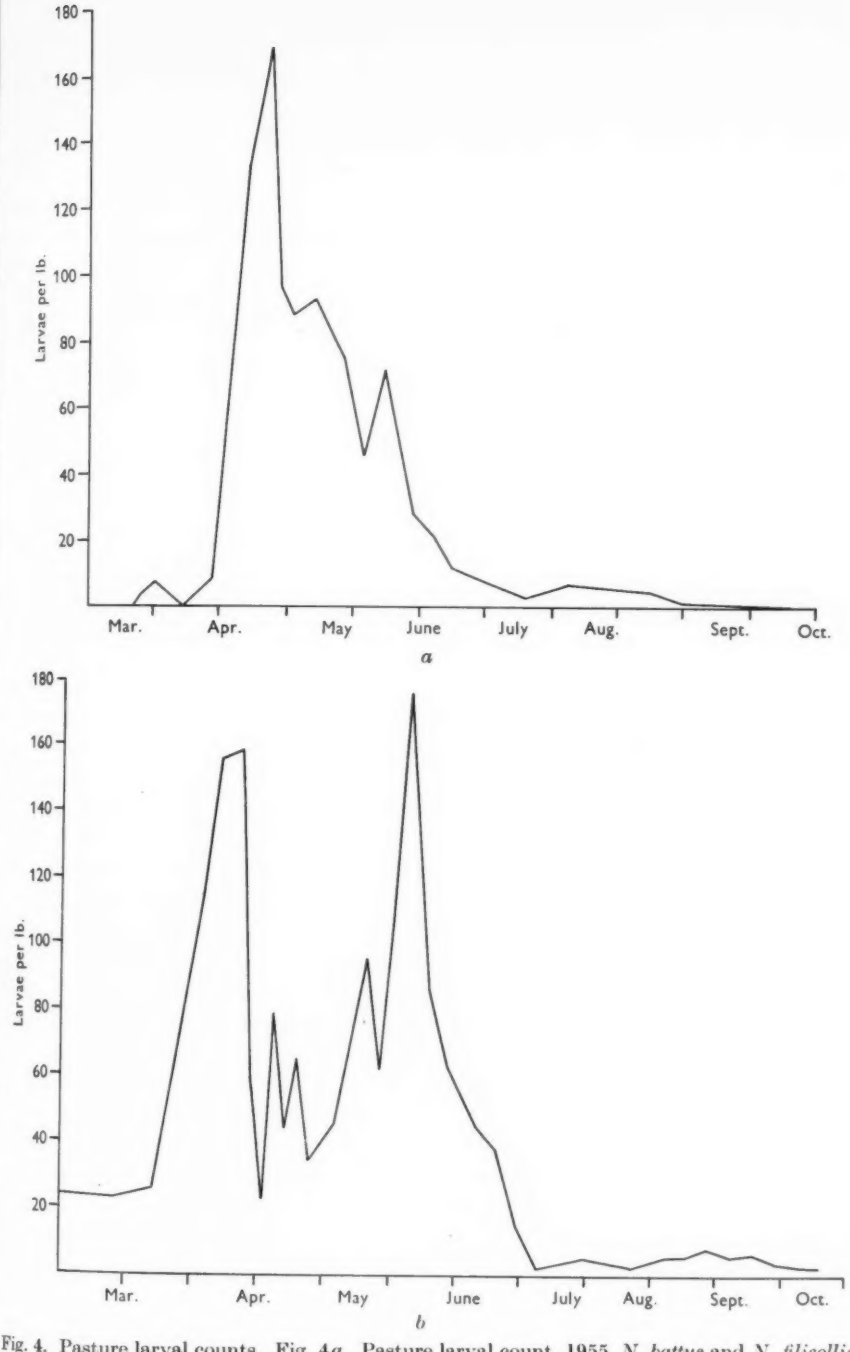
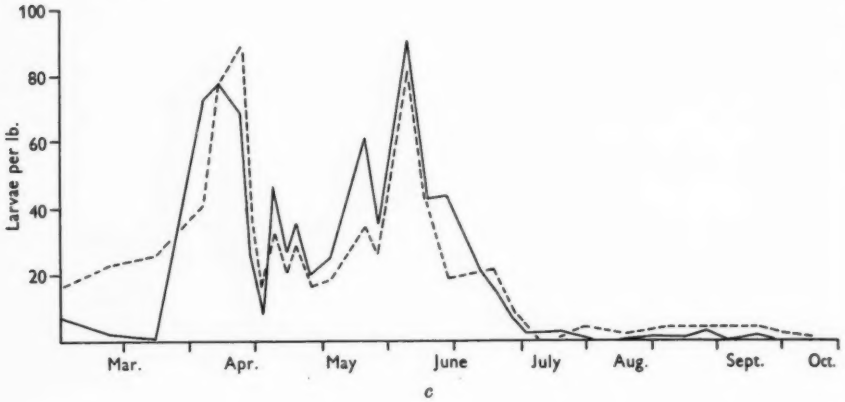


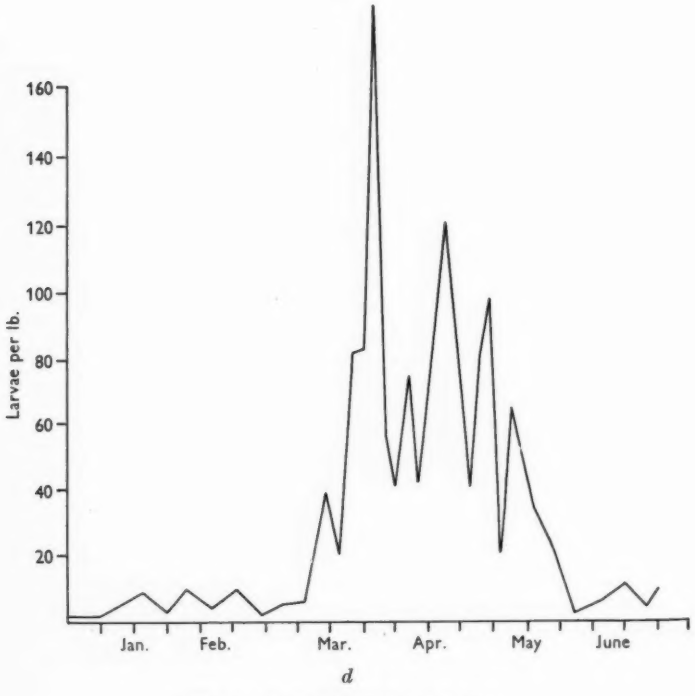

Fig. 4. Pasture larval counts. Fig. 4*a*. Pasture larval count, 1955, *N. battus* and *N. filicollis* combined. Fig. 4*b*. Pasture larval count, 1956, *N. filicollis* and *N. battus* combined. Fig. 4*c*. Pasture larval counts, 1956. ——, *N. battus*; – – –, *N. filicollis*. Fig. 4*d*. Pasture larval count, 1957, *N. battus* and *N. filicollis* combined. Fig. 4*e*. Pasture larval counts, 1957. ——, *N. battus*; – – –, *N. filicollis*.

were present in approximately equal numbers throughout. Very low counts were recorded during the autumn and winter, *N. filicollis* predominating.

At the end of March 1957, the count again rose rapidly to a peak of 200 larvae per pound on 2 April and then declined in a series of subsidiary peaks to a low level in early June. The counts for *N. battus* and *N. filicollis* again followed the same pattern, but in this season *N. battus* predominated, forming 65–80 % of the total count throughout.




*c*




*d*

Fig. 4*c*, *d*. For legend see p. 401.

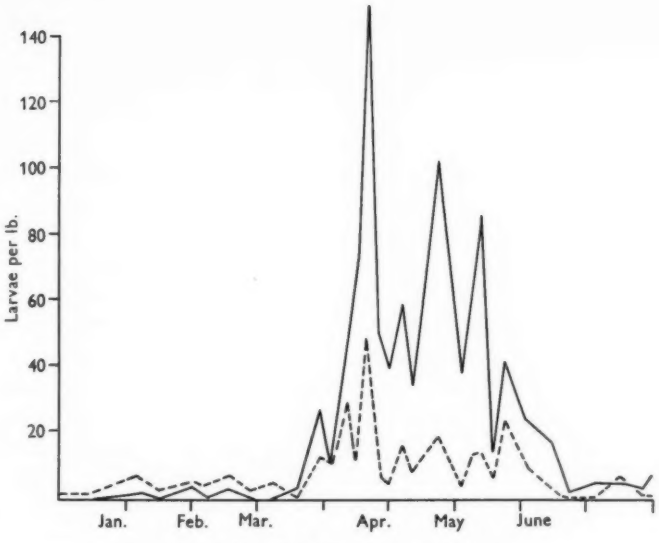



Fig. 4*e*. For legend see p. 401.

## DISCUSSION

The striking feature of these results is the marked seasonal incidence of the *Nematodirus* egg counts in the lambs, and of the third-stage larvae on the pasture. High numbers of larvae were recorded only in April–June of each year, and outside this period only low counts occurred. Similarly, the egg counts in the lambs showed a consistent pattern, rising rapidly to a peak in late April–early June, and falling equally rapidly to a low level in July, this low count persisting until winter, when the count usually reaches zero. Thus the peak of larval population and the peak of egg production are closely related, as would be expected, the latter falling some 4 weeks after the former. The post-mortem records for the lambs confirm that a rapid build-up of the worm burden occurs in early May, reaching a peak in May–June, and thus showing close correlation with the larval and egg-count patterns, although the decline in the worm burden is considerably slower than the decline in the egg count.

The existence of only one period of high larval activity and high worm burdens demonstrated in this work suggests that in these two species only one generation of worms is produced each year. The life cycle from the egg to the mature adult takes 12 months for completion and involves a prolonged inactive pasture phase. Infection in one spring results in a high egg output in May and June, but this contamination does not become available to infect new hosts until the following spring, the worms presumably overwintering as unhatched larvae.

This type of life history, requiring 12 months for completion, and consisting of only one generation per year, has not been described previously in the Family Trichostrongylidae. Thomas & Stevens (1956), in a preliminary report, showed

that *Nematodirus* larvae could survive for at least 11 months on pasture, and that a period of marked larval activity occurred in spring. Stamp, Dunn & Watt (1955) suggested that the lamb infection in spring might be due to a mass hatching of overwintered larvae, but no details of the life history were given. A similar suggestion was made by Tetley (1941) to explain the seasonal incidence of *Nematodirus* infection in lambs in New Zealand.

Crofton (1955) showed that in general the increase in nematode populations in lambs was of the logarithmic type, and that the population increase was the result of a series of self-cures involving a number of generations. Later, a theory (Crofton, 1957) was developed to explain the regular succession of species as a consequence of the life-history pattern, based on the rate of egg production and the time required for the development of each generation. This theory, which could be expressed mathematically, fitted the observed data for all species except *Nematodirus*. A consideration of the rate of increase, as it is shown by *Nematodirus* egg counts, indicated that only one complete generation occurred per season. It was, therefore, concluded that *Nematodirus* was a special case, in which only one generation occurred per year. To explain this, it was suggested that some special factor might be required for the development of each generation apart from the normal temperature and humidity requirements. The more detailed observations made in the present work confirm these conclusions, and the pasture larval counts indicate that the special factor responsible for this type of life history is associated with the prolonged resting phase on the pasture throughout the summer and winter. The development of the pasture stages, the nature of the resting phase, and the stimulation of activity in spring, will be the subject of a further paper.

It is of interest that, by the application of the theory of succession of species, Crofton (1957) pointed out that in theory a second *Nematodirus* generation could occur about September in most years, but he failed to confirm this by field observation. In 2 years of the present study, namely, 1955 and 1956, there was a slight tendency for a rise in the *N. filicollis* egg output to occur in the lambs in September–October, but this was not shown by *N. battus* and was not reflected in the pasture larval count. Moreover, Tetley (1941) found a marked seasonal infection in the autumn in yearling sheep in New Zealand, which suggests the possibility of a second period of infection. Thus, it is possible that, under certain climatic conditions, a second generation can occur, but in Britain this occurs to a very limited extent or not at all. The close agreement between the theoretical deductions made by Crofton and the field observations, provides additional support for the general applicability of Crofton's theory of species succession.

The egg count pattern obtained in this work shows close agreement with the results obtained by Crofton (1955) for *N. filicollis* in south-west England, and by Baxter (1957) for a mixed infection in Northern Ireland. In Scottish hill lambs, Parnell, Rayski, Dunn & Mackintosh (1954) found a similar pattern, but the peak counts occurred slightly later, in July, which was possibly due to the later lambing season. Thus, allowing for variations in lambing times, the season of *Nematodirus* infection in lambs would appear to be remarkably constant in Britain.

The results obtained for the ewes and hoggs in this work indicate that these play

only a small part in the maintenance of infection. Only low egg counts and worm burdens were recorded throughout, and these tended to be restricted to the period May–June, this tendency being more marked in the ewes than in the hoggs. It is of interest that the period of maximum egg counts in the ewes coincided with the period of high egg counts in the lambs. Similar results were obtained by Crofton (1954*a*) working with *N. filicollis* in ewes in south-west England, and, in later studies made by Crofton (1955, 1957), it was noted that *Nematodirus* species in ewes were exceptional in that the infection appeared simultaneously in ewes and lambs.

This may be explained in terms of the marked seasonal activity of the infective larvae of this species. The 'spring rise' in egg count of other strongyles occurs shortly after lambing, and has generally been attributed to a loss of resistance at this time resulting in: (*a*) an increase in egg output of adult worms already present; (*b*) the maturing of larvae whose development has previously been inhibited; and (*c*) the development of newly acquired infections (Morgan, Parnell & Rayski, 1951; Crofton, 1954*a*; Spedding & Brown, 1956). In the ewes sampled in this work the egg counts indicate that for some months prior to lambing the adult *Nematodirus* burden is extremely low or completely nil. The pasture larval counts show that both the numbers of larvae picked up and accumulating in the gut during the winter, and the number available to set up a fresh infection at lambing time, are extremely small. Thus the actual and potential *Nematodirus* burden at this time is so low that it is unlikely to produce a measurable egg count. In April–May, however, some weeks after lambing, the ewes become exposed to an extremely high level of infection, which may partially succeed in overcoming the resistance, and producing a measurable egg count, coinciding with the period of high egg counts in the lambs.

The low incidence of infection in the ewes and hoggs, and the rapid decline in egg counts which occurs in the lambs, indicate the development of a strong resistance to re-infection. Larval worm counts in the hoggs showed that, during March–June, fourth-stage larvae could be recovered from over 50 % of the animals slaughtered, yet adult burdens did not develop, indicating the operation of a strong resistance preventing the further development of the larvae. In addition, a comparison of lamb egg counts in 1956 with the graph of larval activity in that year shows that the marked double peak of larval activity was not followed by a similar double peak of egg output. The second larval peak in June should have caused a second peak of egg output in July, but this did not occur. This again indicates the development of a strong immunity following the initial heavy infection, and shows that this immunity develops rapidly, being present within two months of the original larval invasion.

In the lambs, however, the rapid decline in egg counts is accompanied by a rather slower fall in worm burden. Table 6 shows the individual egg counts of the lambs slaughtered in 1956, together with the post-mortem worm counts. It can be seen that, in lambs killed after the end of May, the egg counts dropped to a low figure, yet quite considerable worm burdens were still present. This suggests that resistance is built up rapidly following the initial infection, which effectively

inhibits egg production and larval development, but does not cause an immediate elimination of the adult worm burden. The results obtained by Baxter (1957) show a similar trend, a rapid fall in egg output being followed by a slower elimination of the worm burden. Stewart (1950) and Soulsby (1957) have demonstrated that depression of egg output can also occur without elimination of the worm burden in the case of other intestinal nematodes.

In the lamb egg counts it was noted that the eggs of *N. filicollis* persisted longer than those of *N. battus*. Furthermore, post-mortem counts showed that in the hoggs the worm burden was almost entirely *N. filicollis*, whereas in the lambs

Table 6. *Lambs* 1956. *Egg counts and post-mortem worm counts*

| Lamb no. | Weekly egg count *Nematodirus* (e.p.g.) 1 May | 8 May | 15 May | 22 May | 29 May | 5 June | 14 June | 20 June | 27 June | 3 July | 10 July | *Nematodirus* worm count | Date slaughtered |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 30 | — | — | — | — | — | — | — | — | — | — | 364 | 4 May |
| 6 | 100 | — | — | — | — | — | — | — | — | — | — | 1,414 | |
| 7 | 100 | 180 | — | — | — | — | — | — | — | — | — | 1,838 | 11 May |
| 8 | 460 | 880 | — | — | — | — | — | — | — | — | — | 5,533 | |
| 9 | 120 | 150 | 250 | — | — | — | — | — | — | — | — | 768 | 18 May |
| 10 | 160 | 750 | 860 | — | — | — | — | — | — | — | — | 3,852 | |
| 11 | 420 | 250 | 760 | 490 | — | — | — | — | — | — | — | 5,736 | 25 May |
| 12 | 260 | 560 | — | 1,100 | — | — | — | — | — | — | — | 5,066 | |
| 13 | 0 | 460 | — | 1,130 | 180 | — | — | — | — | — | — | 10,426 | 1 June |
| 14 | 30 | 910 | 860 | 2,010 | 730 | — | — | — | — | — | — | 4,844 | |
| 15 | 0 | 220 | 680 | 1,400 | 360 | 120 | — | — | — | — | — | 3,564 | 8 June |
| 16 | 910 | 860 | — | 1,050 | 220 | 90 | — | — | — | — | — | 3,403 | |
| 17 | 0 | 520 | — | 1,060 | 1,180 | 400 | 65 | — | — | — | — | 1,720 | 15 June |
| 18 | 30 | 60 | 90 | 580 | 60 | 130 | 230 | — | — | — | — | 3,111 | |
| 19 | 90 | 450 | 630 | 790 | 300 | 65 | — | 0 | 0 | — | — | 3,272 | 22 June |
| 20 | 0 | 0 | 30 | — | 500 | — | 850 | 30 | — | — | — | 12,561 | |
| 21 | 1,050 | 2,600 | 1,600 | 2,160 | 1,180 | 30 | 30 | 30 | 0 | — | 65 | 937 | 10 June |
| 22 | 0 | 30 | 330 | — | 550 | 730 | 65 | 65 | — | 0 | 65 | 2,279 | |

running in the same field, the worm burden was predominantly *N. battus*. Thus the resistance appears to be stronger against *N. battus* than against *N. filicollis*, probably because *N. battus* is the dominant species in this area, the higher numbers of this species resulting in a greater stimulus to resistance than is the case for *N. filicollis*.

A comparison of the proportions of *N. battus* and *N. filicollis* in larval and egg counts over the period 1955–57 shows that, although the proportion of *N. filicollis* eggs passed in 1955 was only 6 % of the total, the proportion of *N. filicollis* larvae present the following spring was 50 %. This declined again over the following season until the proportion of *N. filicollis* eggs passed in 1957 was only 10 % of the total. This variation in proportions may reflect differences in the optimum requirements of the eggs and larvae of the two species, and the difference in distribution of the species in the north and south of England tends to confirm this suggestion. It is of interest that the summer of 1955 was extremely dry, whereas that of 1956 was very wet, suggesting that *N. filicollis* may be more resistant to desiccation.

However, observations over a much longer period would be necessary before any firm conclusions could be drawn.

The variation in *Nematodirus* burden in hill sheep reported by Morgan *et al.* (1951), and by Parnell *et al.* (1954) may be correlated with the life-history pattern demonstrated in this work. Their results differed from those reported above in that low burdens were found in lambs in summer, showing a gradual increase in autumn, and then a marked rise the following year to reach a maximum in late spring, and a subsequent decline to a persistent low level. Lambing takes place from mid-April to mid-May or on hill farms even later. If, therefore, the seasonal larval activity is similar to that recorded for north-east England, i.e. April–early June, then the bulk of the lambs would not graze until after the peak of larval activity, and would only be exposed to a low level of infection, which persists into the autumn. Thus the worm burden would be expected to build up quite slowly over this period. Assuming that the degree of immunity is related to the size of the original infection, this would explain the relative susceptibility of hill hoggs, and the development of heavier burdens in the hoggs the following spring, when they would be exposed to high larval activity for the first time.

*Nematodirus* infection in lambs in New Zealand, as described by Tetley (1934, 1935), follows the same pattern as in Britain, heavy burdens being recorded only in young lambs of 3–6 months of age, in the spring and early summer. Tetley considered that larvae were available in similar numbers throughout the year, and attributed the seasonal rhythm solely to the development of a strong acquired resistance to re-infection. This conclusion was based on the fact that non-resistant (parasite-free) animals became infected with *Nematodirus* species when they were placed on contaminated pasture at any time of the year. The results are, however, open to criticism, because only single sheep were used, and only light infections resulted. In the present work it was found that very low numbers of larvae were present on the pasture for most of the year, so that low infections would be expected in non-resistant animals at any time. It is possible, therefore, that the behaviour of *Nematodirus* species in New Zealand is due to a seasonal larval activity similar to that which occurs in Britain. In yearling sheep, however, a different pattern was found in New Zealand, only small burdens developing in the spring, which were followed by larger burdens in autumn. As noted previously, this suggests the possibility that two periods of larval activity occur under New Zealand conditions, with the result that there were two generations of worms per year.

The life histories of a number of other members of the family Trichostrongylidae have been described in some detail and these follow a basic pattern which is very different from that described above for *Nematodirus*. Development during spring and summer is rapid, the infective stage being reached, under favourable conditions, in 5–15 days and the life cycle may be completed in 4 weeks. Under these conditions the host can harbour a number of generations of any one species during the season, and the build-up of the worm burden is due to repeated re-infection. Survival of eggs and larvae over the winter is very limited, and the major factor in initiating the lamb infection in spring is probably the spring rise in the egg output of the ewes (Crofton, 1955).

The difference between the life history described above for *N. battus* and *N. filicollis*, and the typical trichostrongyle life history, explains a number of unusual features in the aetiology of *Nematodirus* infection. The fact that high *Nematodirus* burdens and disease outbreaks are almost entirely restricted to the period May–June is due to the occurrence of only one generation of worms per year, and to the stimulation by this infection of a marked resistance. The rapid build-up of the worm burden in early spring is due to the fact that a sudden activation of larvae occurs at this time, resulting in the rapid accumulation of large numbers of available larvae, which probably developed to this stage the previous summer, so that no period of development in spring is required. This also accounts for the acute nature of the disease and for the suddenness with which it appears. That the disease tends to be self-limiting, and persists only for about a month, is due to the occurrence of only one generation per year, so that no re-infection occurs even when the flock is maintained on the same pasture for a long period. The severity of the disease may be attributed to the burrowing activity of the developing parasites within the intestine of the host (Thomas, 1959), while the restriction of the disease to the lamb crop is due to the solid immunity produced by the lamb infection, which persists in the adult animal.

This unusual life cycle also explains the failure to control *Nematodirus* infection in this area by traditional methods. These involve the use for lamb grazing of pastures which have been rested for some months during the winter, and are therefore considered 'clean', together with the regular weekly rotation of pastures to break the parasitic life cycle. These methods give a fair measure of control of most of the gastro-intestinal nematodes, for winter survival of the pasture stages is very limited, and weekly rotation reduces re-infection and therefore considerably retards the rate of build-up of the worm burden.

These measures are of no value against *Nematodirus*, for the pasture stages overwinter in large numbers and the worm burden is due to a single massive infection. If lambs are run on a contaminated pasture during the period of larval activity, infection begins immediately and a disease outbreak may occur within 2–3 weeks as the larvae mature. The occurrence of such outbreaks, within a few weeks of moving lambs on to a supposedly clean pasture in spring, was extremely difficult to explain in terms of a normal trichostrongylid life history, but it becomes quite clear when the true life history is understood. Since the introduction of ley pastures, it has become the practice to rest these pastures in winter to give an early bite, and then to reserve them for the lamb crop each spring. Thus the lambs tend to run on the same fields at the same time each year, and in this way an increasingly heavy infection builds up in successive years until a disease outbreak results. This accounts for the high incidence of the disease reported on third- and fourth-year ley pastures.

The application of this knowledge to the control of *Nematodirus* infection is of considerable value. The major source of infection can be traced to the egg output of the lamb crop in May and June and measures can be devised to avoid the use of contaminated fields. Furthermore, the results of pasture sampling show that these fields are quite safe for grazing throughout the year in which contamination

occurred, and are dangerous only for a limited period in the following spring. This period is relatively constant from year to year, extending from the beginning of April to the end of June. Thus the basic principle of *Nematodirus* control is that those fields which carried the lamb flock in May–June of one year may be grazed normally for the remainder of that year, but must not be used for lamb grazing the following spring. Because of their high resistance to infection, older sheep may safely graze these pastures at any time of the year. Low *Nematodirus* burdens would be built up during grazing outside the danger period, but, if this system were followed for a number of seasons, it should be possible to reduce the general level of contamination to a point at which *Nematodirus* would no longer be a problem. There is a practical difficulty in that on a small farm it is not easy to rest productive ley pastures during this period, as this is the season when grass is scarce and all pastures must be fully utilized. However, this difficulty can be overcome by using pastures in alternate years for lamb grazing and for conservation as hay or silage.

## SUMMARY

1. The seasonal incidence of *Nematodirus* infection in sheep was followed by faecal egg counts, post-mortem examination and pasture larval studies.

2. It was shown that only one generation of adult worms was produced each year and that the life history required a prolonged resting stage on the pasture, which took 12 months to complete.

3. The influence of this type of life cycle on the parasitic disease caused by *Nematodirus*, and on its control, are discussed.

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# *LEPTOMONAS* SP., PARASITIC IN THE FLY *LEIA* SP. (FAM. MYCETOPHILIDAE), WITH SPECIAL REFERENCE TO THE METACYCLIC FORMS

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(With 6 Figures in the Text)

The *Leptomonas* described in this paper has been studied principally in the gut contents and faeces of the larva of the fly, *Leia* sp., and in moisture obtained from the decaying vegetable matter within which the larvae grow and pupate. The incidence of infection of the larvae has been found to be almost 100%, only one or two very young specimens being found unparasitized among the hundreds studied.

The remarkable ability of the *Leptomonas* to exist for extended periods under conditions of external exposure, both in the flagellate and non-flagellate condition as will be described, justifies the recording of these notes.

## THE INSECT HOST

The adult fly lays its eggs in very damp mounds of compost. Decaying oak leaves, if sufficiently wet, appear to be particularly suitable for the nourishment and subsequent metamorphosis of the larvae. A favourite site for pupation is on the hollow side of a curled, decaying oak leaf. The adult fly, which is little larger than a mosquito, is not found in dwellings.

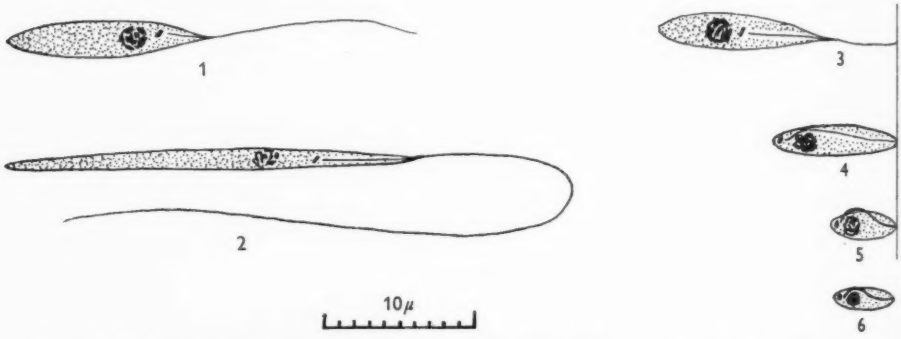
The larvae appear approximately during May at the Cape Peninsula, S. Africa, and may be found for about $2\frac{1}{2}$ months.

## FORMS IN LARVA

The gut of the larva is nearly always infected throughout its length by a great number of leptomonads. They vary in length from short, broad individuals some $14\mu$ long by $2{\cdot}8\mu$ across, with flagella equal to from 1 to $1\frac{1}{2}$ times the body length (Fig. 1), to slender, attenuated leptomonads attaining a length of $26\mu$ and possessing flagella of $1\frac{1}{2}$ or more times the body length (Fig. 2). The nucleus lies within the anterior half of the body and characteristically is close to the kinetoplast, which is situated well forward. The leptomonads do not adhere to the host tissue but are concentrated, for the greater part, near the inner surface of the gut wall. Released into saline, however, they make for, and attach themselves to, fragments of gut tissue by the tips of the flagella.

In the rectum they are smaller; they have very short flagella and are practically incapable of travel. These last forms are passed out with the faeces together with a lesser number of the longer leptomonads with highly active flagella. In hang-drop preparations of larval gut contents the organisms remain active for more

than 48 hr. At no stage is the parasite capable of more than a feeble rate of travel, either within the gut or when liberated into saline, despite vigorous activity of the flagella.




Figs. 1, 2. Leptomonads found in the gut of larvae. Fig. 3. Leptomonad anchored to wall of rectum of adult fly, showing shortened flagellum. Fig. 4. Form with flagellum entirely retracted. Fig. 5. As above, but with shortened body and showing extracytoplasmic path of flagellum. Fig. 6. Spindle-shaped metacyclic form. Note. All the flagellates shown in the figures were stained by Leishman's method.

## FORMS IN PUPA

The infection is intense during the larval phase and becomes even more heavy in the pupa; this is probably because, while multiplication continues as in the larva, the processes of gut elimination and therefore of dissemination of the contained parasites has temporarily ceased. There is no change, however, in the form of the parasite in the pupa, although on rare occasions the Malpighian tubules have been found to be packed with leptomonads which adhered to the tubule walls.

## FORMS IN ADULT FLY

The characteristic form found in the gut of the adult fly is a fat, stumpy leptomonad measuring 14$\mu$ in length with a flagellum as long as the body. Although the flagellum is active there is almost no travel. These forms are often found in rosette formation or adhering to the gut wall.

The rectum is usually lined with adherent leptomonads or with aflagellate, spindle-shaped bodies which have developed directly from these leptomonads. The flagellates attach themselves to the rectum wall by the tips of the flagella and become almost non-motile except for a slight swaying movement of the body. The nucleus and kinetoplast then move posteriorly while remaining close to each other (Fig. 3). Simultaneously with the displacement of these bodies the free flagellum is progressively retracted within the body, which is thereby drawn closer to the rectal wall. When the nucleus has almost reached the posterior tip of the body it is overtaken by the kinetoplast which finally occupies a situation at the extreme tip of the body. By this time the flagellum is entirely retracted and follows a roughly straight path from end to end of the body which remains adherent to

the host tissue (Fig. 4). Subsequently, the organism shortens and finally takes on a spindle-like shape. During this shortening process the flagellum assumes a very characteristic S-shape as it is apparently unable to be accommodated while lying straight within the shrinking body of the parasite (Fig. 5). When suitably orientated it can be seen that the flagellum issues from the surface of the body at a point near the kinetoplast and follows an external path for some distance before becoming intracytoplasmic near the anterior tip. The extracytoplasmic portion appears to be connected to the body by a very minute membrane. Voided forms measure about 5$\mu$ in length (Fig. 6).

## FORMS IN FAECES OF LARVA

The faecal deposits of the larvae contain very large numbers of leptomonads, of which, as has been stated, the greater number are of the short, stumpy type. If the faeces are allowed to lose moisture gradually, as in a moist chamber, it is found that many of the leptomonads take on an ovoid form while the free flagellum becomes retracted within the body. The kinetoplast finally occupies a position at the extreme posterior tip of the body with the nucleus in close proximity, while the flagellum follows an S-bend from the kinetoplast to the anterior tip of the body. This form is identical with the final product found within the rectum of the adult fly, and there seems little doubt that, under natural conditions in the damp compost heap, the defecated flagellates are capable of evolving into the same spindle-like bodies as described in the rectum and faeces of the adult host. This phenomenon, however, does not appear to occur immediately after the faeces are voided under natural conditions. The moisture from a heavily infested compost heap always contains a large number of active flagellate forms of the *Leptomonas*; in addition, it also exhibits a number of the aflagellate, spindle-shaped bodies. The flagellates are mainly of the stumpy type and have not been found to remain alive for more than a few hours under a cover-glass in saline.

If the spindle-shaped (metacyclic) bodies are immersed in saline they revert to flagellate form by a process which is the exact reversal of the retraction phenomenon described as occurring in compost or in the rectum of the adult fly. In 10–30 min. the first vestige of a free flagellum can be made out. For some 15 min., and while it lengthens, the flagellum remains inactive, but when it attains a length of about one-quarter of the body length it begins a periodic flicking from side to side, in a manner similar to flagellating metacyclic forms of *Leptomonas ctenocephali* from the dried faeces of the dog flea (Gibbs, 1947). At first the flagellum remains stationary for about 30 sec. or more after being flicked to one side before it moves in the opposite direction, but gradually the oscillations become more frequent, with concurrent growth of the flagellum, and soon the body is subjected to a steady seesaw movement, but without travel. The organism later attains a length of about 9$\mu$, with a flagellum of body length, and lives for some 12 hr. under a cover-glass. It does not again revert to metacyclic form.

Stained preparations made at various stages of the transformation into the flagellate state show that when the flagellum begins to issue from the anterior tip of the body the remaining portion follows a straighter and an apparently

completely intracytoplasmic path. Until the free flagellum has grown to about one-quarter of the body length there is no change in the location of the nucleus or kinetoplast, but they subsequently become displaced anteriorly. The kinetoplast then moves to the anterior side of the nucleus and finally takes up a position near the anterior tip of the body. Concurrently with these changes there is a considerable increase in the length of the free flagellum.

Active leptomonads have been discovered in damp compost from which all larvae had been removed 2 weeks previously, while spindle-shaped metacyclic bodies, recovered from damp material freed from larvae more than 2 months before, have become active flagellates when immersed in saline.

## FAECAL FORMS FROM ADULT

As has been stated, the walls of the rectum of the adult fly become lined with metacyclic forms and these are passed out with the faecal droplet together with many flagellates. In order to obtain faeces from the flies they were kept under wine-glasses which were inverted over microscope slides. Invariably faecal droplets would be found on the slides. The droplets, however, dried very quickly and it was not found possible to bring about the return of the metacyclic forms to flagellate form in saline.

## DISCUSSION

It is certain that the parasite is passed from fly to larva and from larva to larva by the contaminative method, and it is very likely that infection of a new host can be brought about either by the ingestion of active flagellates or non-motile metacyclic forms. The initial introduction of the *Leptomonas* into a fresh mound of compost must undoubtedly result from faecal contamination by the adult egg-laying fly, but the distribution of the parasite throughout the mass of material is almost certainly accomplished by the larvae.

The ability of the *Leptomonas* to remain in active flagellate condition on the moist surfaces of leaves, etc., is remarkable, even if only in view of the osmotic pressure differences involved. It is considered unlikely that the flagellates found in damp compost 2 weeks after the removal of all larvae had developed from metacyclic bodies as it seems improbable that this would occur without some change in environment or conditions. Under a cover-glass and in saline the stimulus for such reversion is probably the increased salinity and bulk of liquid. It would appear that the faeces of both the larva and the adult fly are capable of disseminating the same spindle-shaped metacyclic form, but in the first case the transformation occurs in damp situations after leaving the rectum of the host. It is not known to what degree the metacyclic forms are capable of resisting desiccation and infecting a new host under natural conditions, but in the laboratory it has not been found possible to induce their development into flagellates once they have been dried.

The phenomenon of the retraction of the flagella, the change in disposition of the nucleus and kinetoplast after the leptomonads have been voided by the host and the subsequent reversal of that process under favourable artificial conditions

was first studied by the writer in *L. cenaei*, parasitic in *Cenaeus carnifex* (Gibbs, 1954). While differing in detail the phenomenon is virtually the same in both cases.

In the present case reversal can take place in saline many weeks after dissemination, provided the flagellate is maintained in sufficiently damp conditions, while in the case of *Leptomonas cenaei* reversal is limited to a period of 24 hr., but during that period the faecal droplet may be allowed to dry.

While the disposition of the nucleus and kinetoplast in the metacyclic form, together with the possible presence of a membrane between the flagellum and the body, suggests trypanosome form, it is not intended to include the organism in the genus *Herpetomonas*, as it is felt that the existence of an actual undulating membrane is too speculative and, excepting in the case of the final product in the rectum of the adult fly, the trypanosome-like disposition of the structures in question occurs only outside the body of the host.

In view of the possibility that the *Leptomonas* may have been previously described in some other host it is not proposed to suggest a specific name.

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# NEW MITE PARASITES OF AFRICAN BIRDS (MYOBIIDAE, CHEYLETIDAE)

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(With 13 figures in the Text)

The majority of the parasitic mites described in the following pages were collected in various provinces of Southern Africa by Dr F. Zumpt of the South African Institute for Medical Research at Johannesburg, to whom I tender my hearty thanks. One species was contributed by Mr P. H. Vercammen-Grandjean from a host taken at Bukavu in the Belgian Congo, while a few others were recorded from birds examined by myself at Pietermaritzburg, Natal. Only one species of this group of mites, *Harpyrhynchus crista-galli*, had previously been taken from a South African bird.

## THE POSITION OF THE GENUS *HARPYRHYNCHUS* IN THE FAMILY MYOBIIDAE

The three genera, *Ophioptes*, *Harpyrhynchus* and *Psorergates* found on reptiles, birds and mammals respectively, form a closely related group; all have the palpi more or less swollen and subglobose, less so in *Psorergates*; all have a single large dorsal shield covering most of the body. *Psorergates*, which occurs only on mammals, is considerably smaller in size than the other two genera and lacks long flagelliform setae on the posterior legs. *Harpyrhynchus* differs from the other two in the curious dorsal armature of the pedipalp and the structure of the posterior legs; in *Ophioptes*, while the armature of the palp is somewhat similar to that of *Harpyrhynchus*, though weaker, the four pairs of legs are similar in construction, all the tarsi being clawless but with cup-like suckers.

The genus *Harpyrhynchus* consists of a rather heterogeneous collection of species differing among themselves in the nature of the dorsal armature of the pedipalp; it also includes, moreover, species such as *H. holopus* Berl. & Trouess., in which the posterior legs are quite differently constructed from those of the remaining species as they have large stout claws and a full complement of segments. The late W. B. Dubinin, before his untimely death in 1958, proposed the genus *Harpypalpus* (1957) to accommodate this species and also that recently described by Fritsch under the name of *Harpyrhynchus longipes* (1954).

### **Harpypalpus** Dubinin

Resembling *Harpyrhynchus* in having the body almost as wide as long, the dorsal surface almost covered by a large shield. Dorsal surface with four long anterior setae, one dorsal and one ventral lateral seta, between legs II and III, but with a number near the posterior margin of scute as well (as in *Ophioptes*). Pedipalps as in *Harpyrhynchus*, short and swollen, two short and one long curved hair

(simple or weakly serrated?) near dorsal apex. Legs short and stout, differing markedly from *Harpyrhynchus* in the two posterior pairs being undifferentiated, without a reduction of segments and with claws similar to the anterior legs. Last and penultimate segments of posterior legs with at least one long flagellum each.

*Type. H. holopus* Berl. & Trouess.

The genera *Harpyrhynchus*, *Psorergates*, *Ophioptes*, *Picobia* and *Syringophilus*, originally included in the family Cheyletidae, were transferred to the family Myobiidae, originally created by Mégnin in 1877, by Baker (1949). Neither Radford (1954) nor Jameson (1955) however have acquiesced in their reception into this family since none of these genera are mentioned by these two authors in their respective revisions of the Myobiidae. The family Myobiidae (Ewing's Myobiinae of 1938) are considered by Jameson as consisting of thirteen genera, *Myobia* and its derivatives, forming a very homogeneous group parasitic on four orders of mammals and on no others.

The five genera mentioned above, having been already separated from the Cheyletidae, should now be also separated from the Myobiidae as defined by Jameson from which they differ in many respects, though like them they are entirely parasitic. They are probably more closely related to the Myobiidae than to the mainly free-living and predatory Cheyletidae.

After the MS. of this paper had been sent away for publication, I heard, through the good offices of Dr M. Lavoipierre, Liverpool School of Tropical Medicine, of W. B. Dubinin's 1957 paper. Dubinin in this work had proposed almost the same modifications to the family Myobiidae as I had done, but had gone even further. In brief he erects a new family, the Harpyrhynchidae, with four genera, *Harpyrhynchus*, *Ophioptes*, *Psorergates* and his new genus *Harpypalpus*; he further divides the family into two subfamilies, the Ophioptinae (*Ophioptes* and *Psorergates*) and the Harpyrhynchinae (*Harpyrhynchus* and *Harpypalpus*). *Picobia* and *Syringophilus* he separates entirely from the genera now placed in the Harpyrhynchidae, under another new family, the Syringophilidae. As this status was, however, proposed by Lavoipierre in a short note published in 1953 (*Trans. R. Soc. Trop. Med.* **47**, 7) the last-named author undoubtedly has priority for the family name.

Although the necessity for subdividing the fairly homogeneous family Harpyrhynchidae, with its four parasitic genera, is perhaps doubtful, I have otherwise accepted Dubinin's systematic modifications in full, and accordingly brought this paper into line with the taxonomic changes proposed by him.

## Family HARPYRHYNCHIDAE Dubinin 1957

### *Key to the genera of Harpyrhynchidae*

1. Legs without claws but with cup-like suckers; on scales of snakes. **Ophioptes**
— At least the two anterior pairs of legs with claws; on birds or mammals. **2**
2. Posterior legs reduced to two or three segments, the last segment clawless, ending in two to three long flagellae; on or under the skin of birds. **Harpyrhynchus**
— Posterior legs similar to anterior, with claws. **3**
3. Size small; legs very short and stumpy, claws minute; on mammals. **Psorergates**
— Size larger, legs normal with large claws; on birds. **Harpypalpus** Dubinin

## Genus **Harpyrhynchus** Mégnin

### *Harpyrhynchus crista-galli* Berl. & Trouess. (Fig. 1)

*H. crista-galli* Berl. & Trouess. 1889, *Bull. Bibl. Sci. de l'Ouest*, 2e Année (9), p. 139.

1♀ and numerous larvae from *Colius striatus*, Pietermaritzburg, collected R. F. Lawrence, June 1958.

*Dorsal surface* as in Fig. 1*a*, the dorsal shield much smaller than in *pectinifer* and *vercammeni*; a smooth seta laterally to the opening of the stigma (not shown

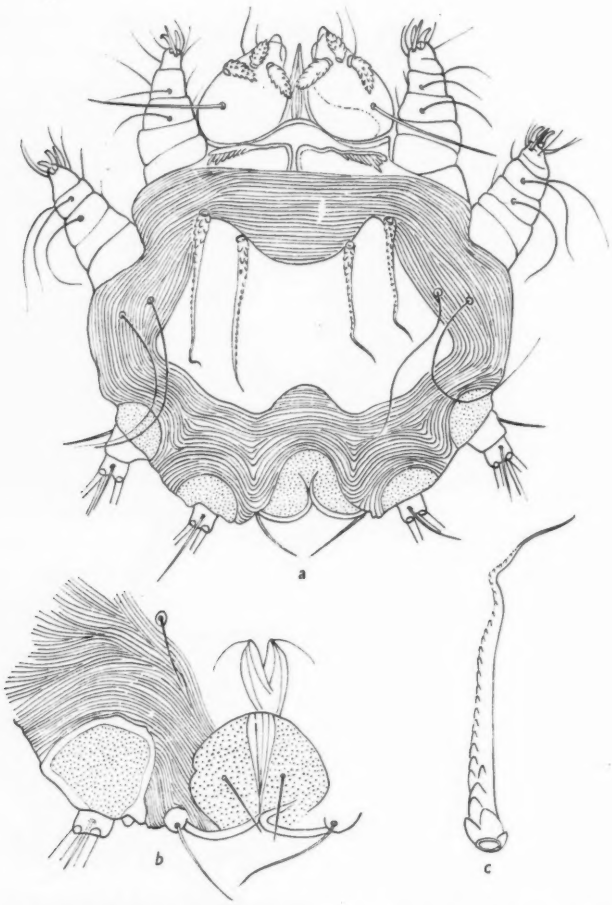


Fig. 1. *Harpyrhynchus crista-galli* Berl & Trouess. ♀: *a*, dorsal surface; *b*, genito-anal region and leg IV enlarged, ventral surface; *c*, anterior flagellum of dorsum enlarged.

in Fig. 1 where it is obscured by the first leg), about as long as those of the dorsal shield but much more slender; four large, curiously modified setae on or near the anterior margin of the shield, aptly described by the authors as covered with

nodules (Fig. 1*c*), so as to resemble a plait (tresse); these setae much thicker at the base than in other species, tapering to a fine point and resembling the plaited thong of a whip lash; two filiform setae laterally instead of one. Peritremes with seven to eight compartments basally, the first two much larger than the others, rounded.

*Ventral surface* differing from *pectinifer* and *vercammeni* in having all the coxal plates clearly defined and marginate, covered with very fine punctures, only 1 with a slender moderate seta, a similar intercoxal pair in the middle at a level half-way between bases of palpi and anterior margin of shield; no ventral lateral seta between coxae II and III, ventral surface thus with five pairs of short setae in all. Posterior apex of abdomen (genito-anal region) and base of leg IV with setation as in Fig. 1*b*, somewhat enlarged.

*Mouthparts.* Pedipalps very wide and swollen, with three short, broad, coarsely spiculate hairs near dorsal apex; in basal half a dorsolateral seta exceeding length of pedipalp.

*Legs* differing from *pectinifer* and *vercammeni* as follows: leg IV with only one short seta in addition to the two long flagella on apical segment; leg III with only one short seta in addition to the three long flagella on apical segment, these segments in IV and III thus with three and four setae-flagella instead of four and five respectively. Longest flagella of posterior legs definitely exceeding width of body, a very little shorter than its total length.

*Dimensions.* Length of body 394$\mu$, width 342$\mu$.

*Larva.* Width of body subequal to length; pedipalps and dorsal shield more or less as in adult but all setae smooth, normal, those lateral to and just below the stigmatal opening more slender than the other four anterior ones. In middle of body one seta just lateral to dorsal shield and one on lateral margin between legs II and III. Leg III represented by a protuberance bearing 3 flagelliform setae, the middle considerably shorter than the others; ventral surface without setae except for 1 at base of leg I.

*Dimensions*: Length of body 189$\mu$, width 185$\mu$.

The species differs completely from any others of the genus in the curiously modified setae of the dorsal surface.

### **Harpyrhynchus pectinifer** sp.nov. (Figs. 2, 3)

*Types.* 3♀♀, 4♂♂, mounted specimens from *Campethera abingoni* (golden-tailed woodpecker), Newington, East Transvaal, collected F. Zumpt, July 1957.

♀. *Dorsal surface.* Body transparent, about as long as wide (Fig. 2*a*). Propodosoma with six pairs of long, rather thick flagella, the anterior pair rising from a small projection just laterally to the stigmatal opening on the anterior margin of the body, the remaining four in a more or less straight transverse row, the medians considerably shorter than the laterals, inserted just in front of the anterior border of the dorsal shield; dorsal surface otherwise without setation except for a stout flagellum near lateral edge of the body between legs II and III; openings of the stigmata very large and conspicuous, with an incompletely closed ring-shaped

peritreme, a main tracheal branch from each meeting in the middle line and then passing side by side forwards and downwards.

*Ventral surface* without shields, the setae arranged as in Fig. 2*b*, most of them

Fig. 2. *Harpyrhynchus pectinifer* sp.nov. ♀: *a*, dorsal surface of type; *b*, ventral surface of a paratype ♀; *c* and *d*, legs IV and III respectively, from below.

fairly long (but much shorter and more slender than those of dorsal surface), except for two on each side of the genito-anal region, which are much shorter, the anterior of the two pairs half as long as the posterior; lateral seta between legs II and III shorter and much more slender than its dorsal counterpart; genito-anal

structures not very clear, weakly chitinized, the anterior (genital) are in some specimens joined to the posterior (anal) region by a median suture.

*Mouthparts.* Rostrum wide, partly covering the bases of the swollen palpi which are rounded, short and wide; palpi apparently with three segments, at the distal apex of the third a stout incrassate spine or hair with subsidiary spicules, directed backwards (Fig. 3*e*); laterally to this a much smaller, rather blunt, spiculate spine, its apex curved somewhat upwards; palp ending in a rather flattened claw, the outer margin curved and provided with a comb-like row of about seven small

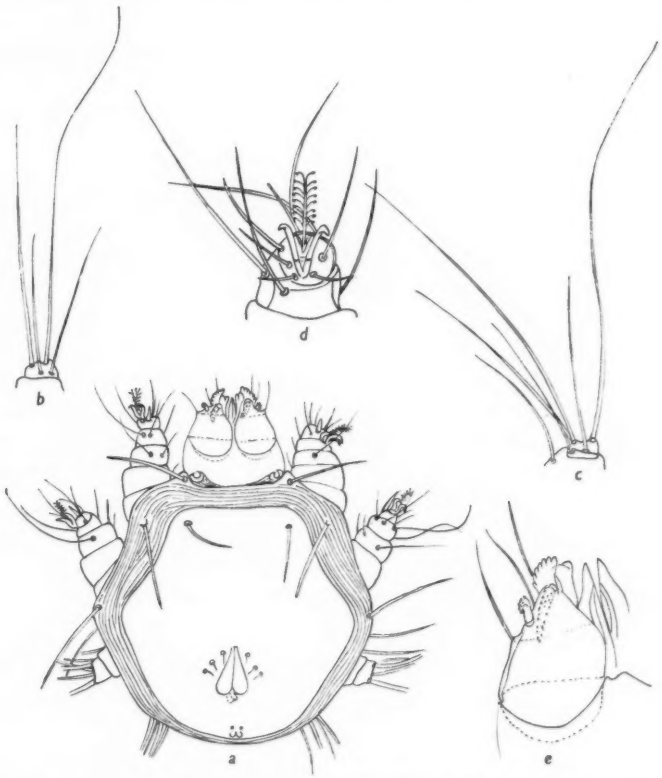


Fig. 3. *Harpyrhynchus pectinifer* sp.nov. *a*, dorsal surface of ♂; *b* and *c*, legs IV and III of ♂ respectively, from below; *d*, apex of leg II of ♀ from below; *e*, palp of ♀, dorsal view.

rounded teeth (Fig. 3*e*); below the claw, the distal ends of the chelicerae visible, their apices blunt and incompletely divided by a cleft; some authors show a small, button-like shield at the base of the pedipalps (Vitzthum, 1929; Banks, 1915), but this is quite absent in the type series and also in a figure by Mégnin (1892) which, however, clearly shows the large opening of the stigmata. Such a shield, though much smaller, is however present on the ventral surface of the gnathosome (Fig. 2*b*).

*Legs.* Anterior legs normal, with a pair of long, stout, equal-sized claws, their

tarsi with a very stout, broad, dagger-shaped sensory seta at the dorso-distal apex, these legs with numerous fine setae on most of the segments, dorsally and ventrally (Fig. 2*a*, *b*); just dorsal to the claws a long feather-hair bifurcate at its apex, with symmetrically arranged lateral barbs, all the remaining setose structures associated with the claw, simple (Fig. 3*d*).

Leg III with only three distinct segments, IV with two; apical segment of leg III with five flagelliform setae, penultimate with only one (Fig. 2*d*). Leg IV (Fig. 2*c*) with only four setae, all on apical segment, arranged in two groups as in leg III; the posterior of the two long, thick, distal setae, longer and thicker than the anterior one in both III and IV, but the difference between them considerably greater in IV than in III. All the setae rising from the ventral surface except the two long distal ones which are inserted on the edge of the apical segment. The longest seta of the posterior legs (posterior seta of leg IV) about equal to length of body including mouthparts.

♂. *Dorsal surface.* Body considerably smaller than that of ♀, dorsal shield continued to posterior margin of abdomen; the antero-lateral flagella distinctly longer than those of the ♀, the two median ones situated well within the borders of the dorsal shield (Fig. 3*a*), instead of just outside it. Genital field situated on dorsal surface just below level of leg III, thus far behind the middle of body, triangular, anteriorly pointed, flanked on each side by a row of three minute, well-separated setae; penis not clearly defined, relatively large, blade-like.

*Ventral surface* with setal arrangement similar to that of the ♀ except that the four shorter setae bordering the genito-anal region are completely absent.

*Legs.* The number and relative length of the setae to each other in legs III and IV similar to those of the ♀; leg IV even less developed than in the ♀ and generally completely covered by the body. The setae of the posterior legs differing from the ♀ only in being relatively much longer, the longest seta of both legs III and IV considerably exceeding the length of the body (Figs. 3*b*, *c*).

The sexes can be distinguished by the following characters:

| | Males | Females |
|---|---|---|
| 1. | Body smaller | Body larger |
| 2. | Longest seta of posterior legs considerably exceeds body length | Longest seta of posterior legs equal to body length, |
| 3. | Antero-median flagella situated on dorsal shield | Antero-median flagella situated just in front of dorsal shield |
| 4. | Genital opening dorsal, flanked by three pairs of minute setae | Genital opening ventral, flanked by two pairs of much longer setae |

*Dimensions.* ♀, length of body 280–312$\mu$, width 220–254$\mu$; ♂, length of body 230$\mu$, width 180$\mu$.

All specimens taken from alcohol in which the host had been preserved so that nothing is known about the ecology of the mite.

**Harpyrhynchus vercammeni** sp.nov. (Fig. 4)

*Types.* 2♀♀ from the black-chested coucal, *Centropus toulou grilli* (L. 23554/1), Luvungi, Ruzizi, Belgian Congo, collected P. G. Vercammen-Grandjean, May 1954.

*Dorsal surface.* Differing from *H. pectinifer* in the dorsal shield not being as wide in relation to its length, the much longer flagellae of the anterior dorsal surface, which are almost as long as the dorsal shield and certainly considerably thicker in their basal halves than those of *pectinifer*, appearing to be somewhat flattened (Fig. 4*a*).

Fig. 4. *Harpyrhynchus vercammeni* sp.nov. ♀: *a*, dorsal surface; *b*, dorsal view of palp.

*Ventral surface* with the same general arrangement of the setae as in *pectinifer*, the setae of the posterior legs definitely thicker and longer than those of *pectinifer* but arranged in a similar pattern. Anterior legs relatively longer and more robust than in *pectinifer*, the dagger-like sensory spine at the dorsal apex of the tarsi not

as broad and somewhat longer. The longest flagellum of both legs III and IV exceeding the total length of the body.

*Pedipalps* differing markedly in having two similar thickened fleshy hairs on the dorsal surface near apex with large blunt accessory spicules; these hairs much larger than in *pectinifer* and subequal, or the lateral hair even a little larger than the mesial one instead of being considerably smaller (*pectinifer*); the details of the shape of the apex of chelicera (cf. Figs. 4*b* and 3*e*) different in the two species.

*Dimensions.* Length of body 320$\mu$, width 260$\mu$.

The two species of *Harpyrhynchus* described above differ from the genotype, *H. nidulans*, in being almost as wide as long, the sexual opening of the ♂ lying in the posterior instead of the anterior half of dorsal surface and in being considerably smaller in size. They resemble in general two species described by Fritsch (1954), *capellae* and *tracheatus*.

### **Harpyrhynchus ovalis** Fritsch

1♀ with eggs, from the house sparrow, *Passer domesticus* (introduced), Pietermaritzburg, June 1958.

This single specimen, taken from the head of the host, agrees well with Fritsch's description and figures. There are now three species known from this host and *pilirostris* Berl. & Trouess. (1889), also taken from the head of the sparrow, may prove to be identical with Fritsch's *ovalis*. The description of *pilirostris* is very brief and inconclusive and no figure is given.

Few hosts have been examined for species of *Harpyrhynchus* and the mites are not easy to find; in my opinion also only a limited number of birds are parasitized by this mite, nevertheless the number of species in the genera may eventually prove to be very great. A slide kindly loaned me by Dr G. Owen-Evans of the British Museum of Natural History carries a single ♂ specimen from an owl which, though related to *pectinifer*, appears to be a new species; on the basis of this single specimen, however, I have not named or described it.

Fritsch has recently (1954) given an excellent key to the European species. So many European birds however have been introduced into Africa or are seasonal migrants there that I have ventured to include all the old-world species in the following key with the exception of the dubious *H. tabescentium* (Berthold), the two American species *H. longipilis* Banks and *H. brevipes* Ewing being thus omitted. It is partly adapted from the key given by Fritsch (1954, pp. 179, 180).

*Key to the old-world species of Harpyrhynchus*

1. Pedipalp long, cylindrical, with three short, smooth, spine-like setae at apex.
**cylindripalpus** Fritsch

— Pedipalp short, wide, subglobose, with two or three longer, serrated, backwardly directed hairs. 2

2. Modified hairs of pedipalp as long as the appendage itself, with weak serration, posterior legs of ♀ inserted at about the middle of the body.
**pilirostris** Berl. & Trouess.

— Modified hairs of the pedipalp considerably shorter than the appendages, usually thick with coarse serrations; posterior legs inserted behind middle of body. 3

3. Legs III and IV of ♀ with only two or three segments. **4**
— Legs III and IV of ♀ reduced even further. **9**
4. Dorsal setae with serrations or nodules. **5**
— Dorsal setae quite smooth. **6**
5. Apex of leg III with about seven setae; dorsal setae of abdomen with fine sharp serrations. **nidulans** Mégnin
— Apex of leg III with four setae; dorsal setae of abdomen very thick in basal half, with round nodules like a plait. **crista-galli** Berl. & Trouess.
6. Pedipalp dorsally with two slender, weakly serrated hairs, its apex with a forwardly directed toothed claw. **pectinifer** sp.nov.
— Pedipalp dorsally with three serrated hairs, with or without a serrated claw. **7**
7. Pedipalp dorsally with three weakly serrated hairs, no conspicuous apical claw. **capellae** Fritsch
— Pedipalp with three much more strongly serrated hairs, a conspicuous apical claw. **8**
8. Pedipalp with three very wide coarsely serrated hairs, apical claw smooth **vercammeni** sp.nov.
— Pedipalp with three moderately thick, not strongly serrated hairs, apical claw weakly serrated. **tracheatus** Fritsch
9. Legs III and IV with 1 segment, anterior legs reduced, body much longer than wide. **ovalis** Fritsch
— Legs III and IV reduced even further, or situated on dorsal surface. **10**
10. ♀ much wider than long; anterior legs lateral, short and thick; leg III reduced and flask-shaped, IV a thickly spined dorsal plate; ♂ normal with enormous dorsal penis. **monstrosus** Fritsch
— Anterior legs of ♀ submedian and strongly reduced. **11**
11. Hairs of pedipalp slender, weakly serrated, no toothed apical (fussförmig) claw; legs III and IV with 1 segment, implanted laterally. **plumaris** Fritsch
— Hairs of pedipalp thick, short, and coarsely serrated; a toothed (foot-shaped) apical claw; leg III of ♀ a minute dorsal papilla, leg IV absent. **reductus** Fritsch

## Family Syringophilidae Lavoipierre 1953

### Genus **Syringophilus** Haller

### **Syringophilus zumpti** sp.nov. (Fig. 5)

*Types.* 2♀♀ from the ring-necked dove *Streptopelia capicola*, at Colesberg, Cape Province, collected by F. Zumpt, December 1953. The two specimens have been dechitinized in caustic potash and have lost a number of the body setae; nevertheless, these individuals differ from two specimens, of *S. pectinatus* and *S. totani* respectively, loaned me by the British Museum through the kindness of Dr G. Owen Evans.

Differing from these two species in the following details: body smaller but the palpi and legs much thicker and relatively shorter, the palpi reaching forward well beyond the apex of the anterior legs; the dorsal shields appear to be very ill-defined. The larger setae of the dorsal surfaces of palpi and legs much thicker in basal half than in either *pectinatus* or *totani* (Figs. 5*a*, *d*). Posterior apex of abdomen with only one pair of long setae (other species with at least two pairs).

The claws of the posterior legs differ radically from those of the other species examined in being very unequal, the anterior ramus being thick, well curved, and

much shorter than the posterior one, which is long, slender and weakly curved; the anterior claws are equal in length and much thicker than in the other species (Fig. 5*b*), also thicker than those of posterior legs. The two characteristic feather-like hairs behind the claw on dorsal surface (see Hirst, 1922, p. 58, fig. 42*a*) appear to be absent, the two combs of the empodium with very long, fine setae (Fig. 5*c*).

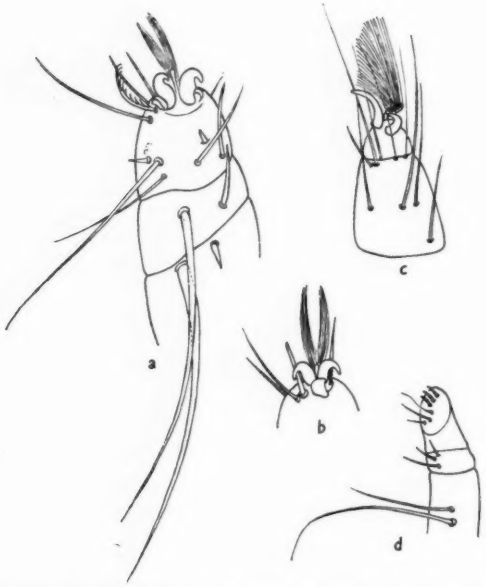


Fig. 5. *Syringophilus zumpti* sp.nov. ♀: *a*, apex of leg I, dorsal view; *b*, apex of tarsus II, dorsal view; *c*, apex of leg IV, dorsal view; *d*, palp, dorsal view.

*Dimensions.* Total length 652$\mu$.

The single specimen in the British Museum collection diagnosed as *S. totani*, from the Ruff, *Philomachus pugnax*, may not actually belong to this species, the body length being almost twice that given by Vitzthum for *S. totani* (1929, p. 53).

The following are the main characters observed: pedipalps not reaching as far as the apex of the anterior legs, long, slender and cylindrical; dorsum, legs and palpi with slender, weak setae; two pairs of long setae at posterior apex of abdomen, one dorsal, one ventral; between these two stout but quite short setae bordering the genito-anal opening on each side. Posterior claws long and slender, much longer than in *zumpti* and *bipectinatus*, strongly curved, equal; the feather-like hairs of legs III and IV very well developed, inserted dorsally half-way between base of tarsus and base of claws, less well developed and much shorter in leg II; in I these hairs almost simple, with one or two lateral cilia. Peritremes with the lateral branch consisting of eight or nine compartments (six or seven in *bipectinatus*, four in *columbae*).

*Dimensions.* Length of body 1230$\mu$.

In its size and the palpi not surpassing the apex of the anterior legs, this form

resembles *columbae* Hirst. The following species of *Syringophilus* have been described: *major* Trouessart, 1895; *bipectinatus* Heller, 1880; *totani* Oudemans, 1904; *helleri* Oudemans, 1904; *columbae* Hirst, 1920; and *zumpti* sp.nov.

Family CHEYLETIDAE

Genus **Neocheyletiella** Baker

*Neocheyletiella* Baker, 1949, *Proc. U.S. Nat. Mus.* **99**, 271.

**Neocheyletiella subquadrata** sp.nov. (Figs. 6*a*, *b*, 7)

*Types*, 4♀♀, 3♂♂, from *Melittophagus pusillus* (little bee-eater), Kanji, Bechuanaland, collected F. Zumpt, July 1956.

♀. *Colour* yellow with a brownish tinge.

*Dorsal surface* as in Fig. 6*a*; one anterior shield with two anterior and one posterior pairs of setae, two further pairs inserted just off the shield, the median anterior pair short, dagger-shaped, fairly wide basally, much shorter than the posterior median pair. The general setal pattern as in *N. chanayi* (Berl. & Trouess.) as figured by Baker (1949, p. 274, pl. 6, figs. 11–13), except that two of the pairs are situated just off instead of on the shield, no suture visible behind the shield; the setae of dorsal surface are in general comparable with those of *chanayi*, though in most cases much longer, the posterior end of the body with four long and four more moderate setae.

*Ventral surface* as in Fig. 6*b*, base of coxa II with a very distinct, blunt, and slightly hooked, smooth process, resembling a large tooth and considerably more chitinized than remainder of coxa; genito-anal opening with one pair of anterior, three pairs of flanking moderate setae, in addition two more mesial shorter pairs at the posterior apex of the opening.

*Mouthparts.* Gnathosoma as in Fig. 7*c* (of male), strongly resembling that of *N. chanayi* as figured by Baker (*loc. cit.* pl. 6, fig. 11), the rostrum, however, narrower; Baker describes the palpal claw as small in his key but I should consider the claw, both of *subquadrata* and his *chanayi*, as figured, robust or even large; the dorsal setae with considerably longer accessory cilia than the ventral and lateral ones, basal segment of palp with two conspicuous simple setae ventrally; basal plate of rostrum with a moderate seta on each side ventrally; the two branches of the peritreme (partly visible through the dorsal epidermis) with six or seven segments.

*Legs.* All claws very robust, anterior legs with very stout, strongly curved sharp claws, shaped distally like a bird's beak, between them an empodium with a fairly thick shaft from which rise two comb-like rows of fine setae, apparently projected at different angles, in general resembling Baker's figure (*loc. cit.* pl. 6, fig. 5) of *canadensis* (Banks); anterior tarsi with a very strong angular projection near dorsal apex, hollowed out in front of this as in Fig. 7*d* (of male), the projection much reduced in the posterior legs, the upper surface of the tarsus sloping gradually downwards in these. Tarsus II with a short, rod-like, blunt-tipped, sensory spine at dorsal apex, that of I much longer and pointed. Legs I and II with a short

feathered hair at the inner apex of second segment, the barbs much longer than those of the remaining setae; posterior legs without such a hair.

♂. *Dorsal surface* as in Fig. 7*a*, the anterior plate ill-defined but much larger than in the ♀, the setae arranged similarly but all of them situated on the shield;

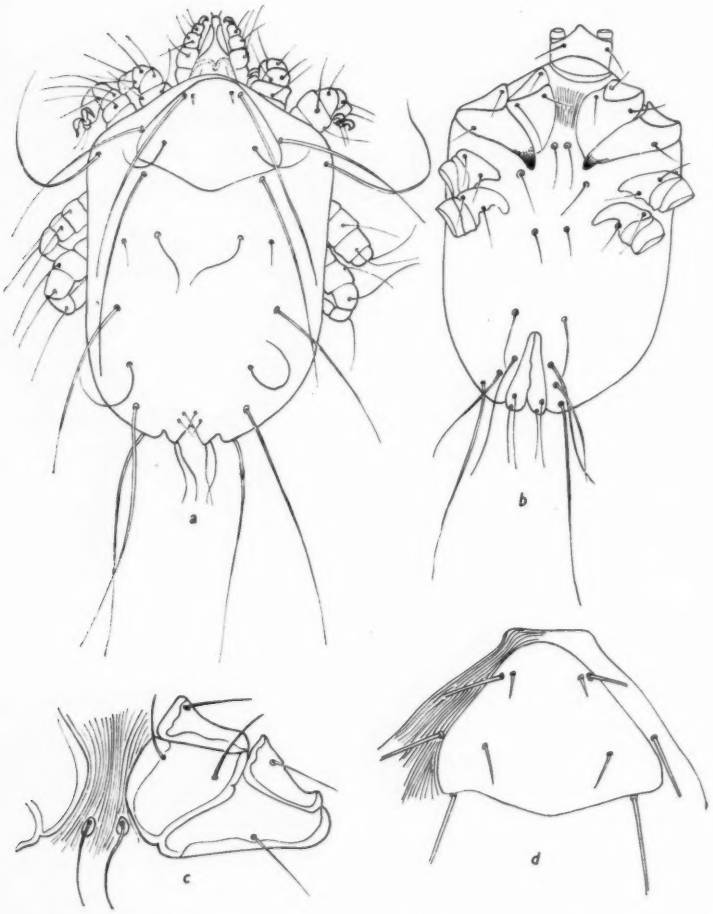


Fig. 6. *Neocheyletiella subquadrata* sp.nov. ♀: *a* and *b*, dorsal and ventral surfaces. *Neocheyletiella transvaalica* n.sp. ♀: *c*, coxae I and II enlarged; *d*, anterior dorsal shield.

anterior median setae not short and dagger-shaped but thread-like and fairly long (about as long as the ventral inter-coxal pair), a little longer than the posterior median pair which are also thread-like; genito-anal opening dorsal, with setation as in Fig. 7*a*. Anterior to the genital opening but beneath the epidermis through which it can be partly seen, an elongated narrow chitinized structure reaching forwards to about the middle of coxa IV, this being probably the penis.

*Ventral surface* as in Fig. 7*b*, differing from the ♀ in having the epimera of coxa I subcontiguous, coxa II without a hooked chitinous process at its base, either entirely without or with a very slight protuberance; the setae of posterior coxae and those on the abdomen posterior to them, much longer than in ♀. Gnathosoma (Fig. 7*c*) and legs not differing in any way from the ♀.

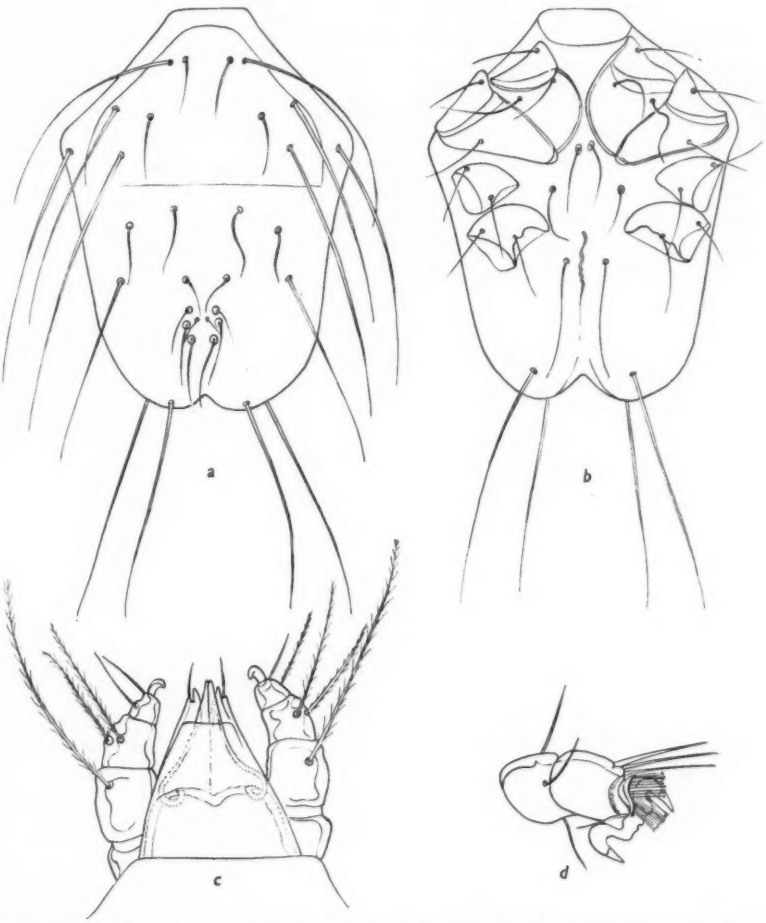


Fig. 7. *Neocheyletiella subquadrata*, sp.nov. ♂: *a* and *b*, dorsal and ventral surfaces, appendages omitted; *c*, gnathosoma from above; *d*, apex of leg II from above.

*Dimensions.* ♀, length of body 480$\mu$, width 275$\mu$. ♂, length of body 300$\mu$, width 225$\mu$.

In addition to the detailed structures described above, the ♂ differs from the ♀ in general in the genito-anal opening being situated dorsally, its distinctly smaller size and in having the body distinctly wider anteriorly, instead of more or less quadratiform.

**Neocheyletiella transvaalica** sp.nov. (Figs. 6*c*, *d*, 8*a*, *b*)

*Types*, 2♀♀ from *Melittophagus pusillus*, Mabelikwa, Transvaal, collected F. Zumpt, January 1957.

This species of *Neocheyletiella* is similar to *subquadrata* in its general form and size but differs as follows: anterior shield of ♀ a little wider, all the four median spines dagger-shaped, broad at their bases and short, the anterior a little shorter than the posterior pair (Fig. 6*d*); the four pairs of long lateral setae posterior to

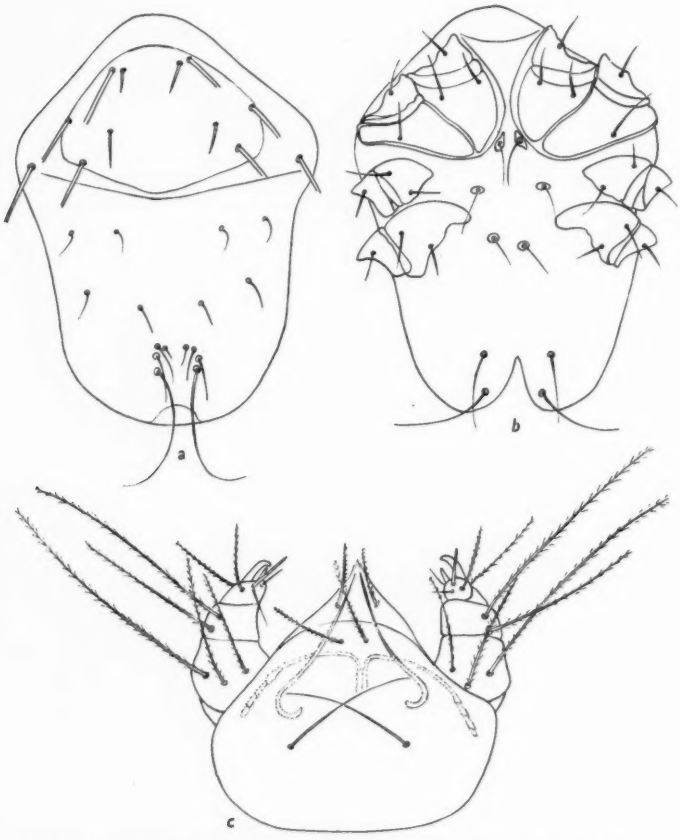


Fig. 8. *Neocheyletiella transvaalica* sp.nov. ♂: *a* and *b*, dorsal and ventral surfaces, appendages omitted. *Neocheyletiella faini* sp.nov. *c*, gnathosoma of ♀, ventral view.

the dorsal shield are relatively a little longer and stouter. Coxa II (Fig. 6*c*) has no hooked chitinous process at its base but is shaped as in the male of *subquadrata*; ventral surface otherwise as in the type of *subquadrata*.

*Dimensions*. Length of body 500 $\mu$, width 274 $\mu$.

Several females from the blue-cheeked bee-eater, *Merops persica*, Mabelikwa,

Transvaal, agree in all particulars with this form. A single ♂ from *Merops apiaster*, taken at the same locality and date, cannot, I think, be separated from this species. It differs markedly from the ♂ of *subquadrata* in the following particulars.

The two median setal pairs of the dorsal shield are short and dagger-like (Fig. 8*a*); the first transverse row of setae posterior to the shield are much shorter, the lateral setae of the second transverse row are quite short, subequal to the medians instead of being much longer (cf. *subquadrata*, Fig. 7*a*).

The arrangement and number of setae on the genito-anal field are similar but the third (posterior) pair are relatively much longer than the others; the two pairs of long posterior setae of *subquadrata*, one dorsal, one ventral, are absent; instead two moderate ventral pairs are present. On the ventral surface (Fig. 8*b*), the anterior intercoxal setae touch the epimera; the third inter-coxal setae are much shorter.

*Dimensions.* Length of body 301$\mu$, width 225$\mu$.

The differences between the females of these two closely related species are slight, but those separating the males are considerably more pronounced.

**Neocheyletiella faini** sp.nov. (Figs. 8*c*, 9, 10)

*Types*, 1 holotype ♀, 1 allotype ♂, and 15 cotype ♀♀ from *Cossypha dichroa*, Mossel Bay, Cape Province, collected F. Zumpt, December 1953.

*Colour* light yellow, usually almost completely transparent.

♀. Body as in Fig. 9, seen from above, the body very thin and flattened in mounting so that it is difficult to distinguish the setation of the dorsal surface from that of the ventral.

*Dorsal surface.* One fairly large dorsal anterior shield which is apparently separated into two quite distinct oval lateral shields by a median bridge. Setation of dorsal surface as in Fig. 9, the setae fine, thread-like, much finer than in *subquadrata* though not as long, all of them more or less barbed. Dorsal surface agreeing with Baker's figure of *N. chanayi* (*loc. cit.* 1949, pl. 6, fig. 12), in having two short setae on each side behind the suture, these springing from small oval plaques about as large as those of the intercoxal setae on ventral surface. Body relatively much larger than in *subquadrata*, the legs and mouthparts much smaller relative to size of body. All setae of body, legs and mouth-parts more or less barbed.

*Ventral surface.* As in Fig. 10*a* (posterior half) the epimera of anterior coxae widely separated, the anterior intercoxal setae situated on small plaques (as in Fig. 10*c* of ♂).

*Mouthparts.* Pedipalp and rostrum as in Fig. 9, not differing markedly from those of *subquadrata*, setation of palpi and rostrum seen from below as in Fig. 8*c*.

*Legs.* Anterior leg surpassing by a little the apex of palpi, posterior legs not reaching posterior end of body. Setae of legs much finer than in *subquadrata*, tarsi without a conspicuous projection at dorsal apex which is truncate, the claws joined to the tarsus by a slender pedicel which is longer in posterior than anterior legs; between the claws, which are much more slender than in *subquadrata*, the

rod-like pulvillus projects perpendicularly, giving off 11–12 forwardly directed knobbed hairs. Sensory rod at the dorsal apex of tarsus I pointed and long, in II shorter, curved and blunt-tipped; a short blunt sensory rod at apex of pretarsal segment in I, this rod dagger-shaped, longer and pressed flat against the segment in II.

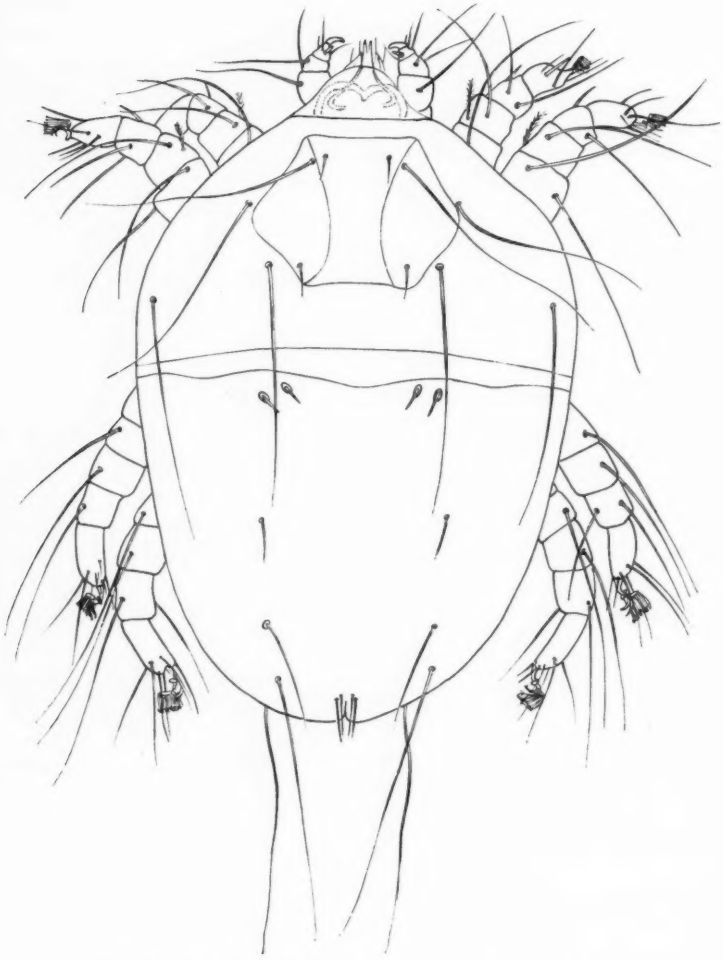

Fig. 9. *Neocheyletiella faini* sp.nov. ♀; dorsal surface.

♂. *Dorsal surface.* The body of male as in *subquadrata*, much smaller and differently shaped from ♀, pyriform, the anterior half distinctly wider than posterior; the dorsal shield (Fig. 10*b*), somewhat differently shaped from that of ♀, hysterostoma without setae between the two pairs of short setae in the middle and those at posterior apex of abdomen.

*Ventral surface* as in Fig. 10*c*, the epimera of anterior coxae not subcontiguous

but only a little nearer to each other than in the ♀. Setae of palpi, legs and body all ciliated as in ♀.

*Gnathosoma* as in ♀.

*Legs* as in ♀ but the posterior a little longer, leg IV surpassing the posterior apex of body by a little, leg I reaching the apex of palpi. Claws of leg II as in Fig. 10*d* seen from above.

*Dimensions:* ♀, length of body 548 $\mu$, width 400 $\mu$; of ♂, length 376 $\mu$, width 266 $\mu$.

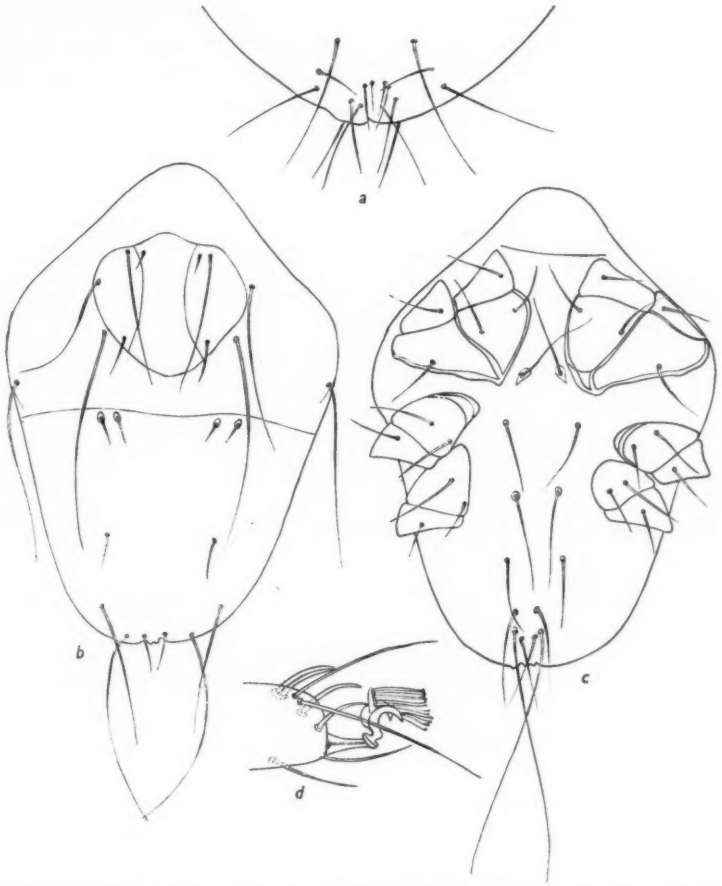


Fig. 10. *Neocheyletiella faini* sp.nov. *a*, genito-anal region of ♀, ventral view; *b* and *c*, dorsal and ventral surfaces of ♂, appendages omitted; *d*, apex of leg II of ♂, dorsal view.

## **Ornithocheyla** gen.nov.

♀. Body large, oval, mouthparts relatively small; palpi short, thickset, 4-segmented, closely pressed to the sides of the rostrum, as in *Neocheyletiella* without comb-hairs, the apical claw small. One small dorsal shield anteriorly. Ventral surface peculiar in having only coxa I defined by a narrow marginate rim (Fig. 12*a*),

the remaining legs completely lacking coxal plates, epimera of leg I widely separated. Body, except for dorsal shield and anterior coxae, covered with fine close striations. Legs short and stout, the anterior considerably thicker than the two posterior pairs, the anterior and posterior pairs hardly surpassing the anterior and posterior extremities of the body respectively; all tarsi with stout, strongly curved claws; leg I with short setae and only one weakly ciliated hair at base of claws, legs II–IV with long setae and two conspicuous strongly ciliated feather-like hairs, one on each side at base of claw.

♂. Penis very conspicuous, more than half length of body, straight, blade-shaped, emerging through a dorsal opening just behind anterior shield; body much smaller, pyriform, the legs longer, the anterior shield differing in shape from that of ♀, the epimera of leg I narrowly separated; all remaining structures resembling those of the ♀.

*Type*. *Ornithocheyla megaphallos* sp.nov.

**Ornithocheyla megaphallos** sp.nov. (Figs. 11–13)

*Types*, 2♂♂, 5♀♀, from *Estrilda erythronotos* (black-cheeked waxbill), Debeete, Bechuanaland, collected F. Zumpt, July 1956.

*Colour* light yellow, the penis of the ♂ much darkened.

♀. *Dorsal surface* as in Fig. 11, all the long setae of body, legs and mouthparts smooth, or very weakly ciliated, the setae of the body springing from small round plaques. Anterior shield unusually small, cordiform, not carrying setae; lateral setae moderately long, finely tapering, filiform, only slightly thickened at their bases, the median pairs shorter.

*Ventral surface* as in Fig. 12*a*; no intercoxal setae, the most anterior of the median pairs situated at the level of apex of coxae I; only leg I with a defined coxal plate, carrying two setae as in *Neocheyletiella*; genito-anal opening at posterior apex of abdomen.

*Mouthparts* with the details as seen through the rostral plate much less clear than in *Neocheyletiella* but of the same type as in other Cheyletid genera, the claws of the palpi directed inwards; peritremes of the same general type as in *Neocheyletiella*, the lateral branches from the peritremes apparently divided into six or seven very large compartments conspicuously wider than long, resembling the antennae of some Coleoptera. In general the pedipalps much shorter, the rostrum relatively wider and shorter than in *Neocheyletiella*. Ventral surface of gnathosome with setation, as in Fig. 13*d*, of the ♂.

*Legs* as in Fig. 12*b*, *c*. Leg I differently armed from legs II–IV, the setae of the various segments much shorter, the fringes of the ciliated hairs on the tarsi much reduced; legs II–IV with a very long dorsal seta on second segment from base which is much reduced in leg I (Fig. 11), these legs also with a conspicuously ciliated short hair on each side at dorsal apex of tarsus (only one in leg I). A strong rod-like perpendicular empodium between the claws of all legs bearing two to four long, slender, apically cleft hairs. Tarsus I with a very thick truncate projection at its dorsal apex (about half as deep as the tarsus itself), bearing three long, thick

sensory rods; remaining legs with the dorsal protuberance much reduced and without these structures (cf. Fig. 12*b*, *c*).

*Dimensions.* Length of body 553$\mu$, width 331$\mu$.

♂. *Dorsal surface.* Body wider anteriorly than posteriorly, widest at the sub-angular lateral projections between legs II and III. Anterior scute moderate,

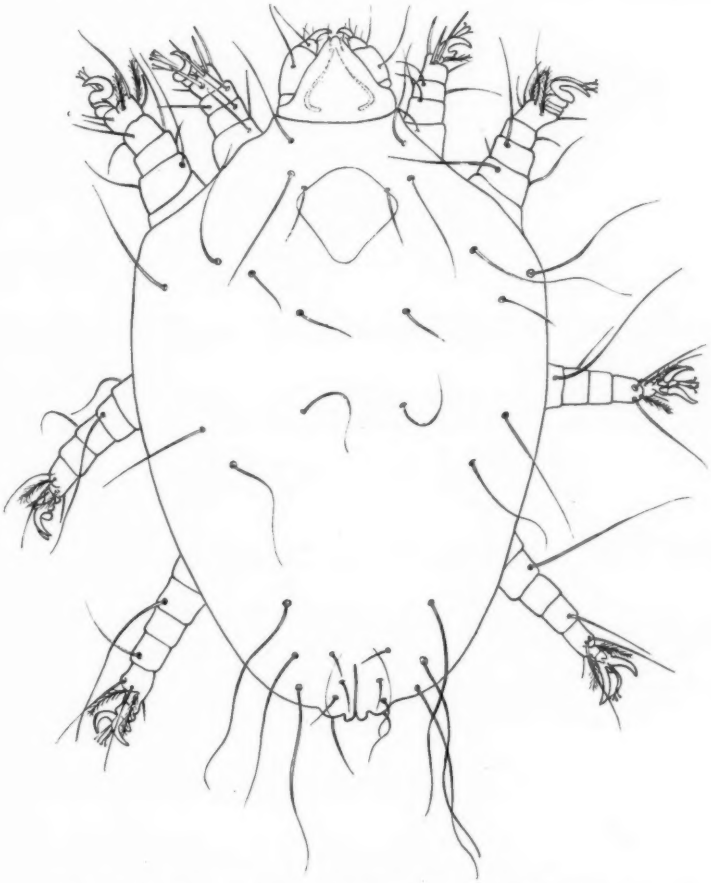

Fig. 11. *Ornithocheyla megaphallos* sp.nov. Dorsal surface of ♀.

wider behind than in front (the opposite of the ♀), with two pairs of short setae; lateral setae of dorsal surface longer than in the ♀ relative to the size of the body. Penis very conspicuous, with dark chitinization very clearly visible through the skin of dorsal surface, long and straight, the sharp point protruding through an opening at the apex of a cone projecting forwards over the anterior dorsal shield (Fig. 13*a*, *c*). Apart from this opening there seems to be no other 'anal' opening either dorsally or ventrally.

*Ventral surface* as in Fig. 13*b*; coxae similar to those of the ♀, only I with

a marginate plate, the epimera narrowly separated. The four pairs of intercoxal setae distinctly longer than those of the ♀, the lateral pairs also relatively if not absolutely longer. Apex of penis reaching to the anterior seta of first coxa or a little below it.

*Mouthparts.* Seen from above not differing from those of the ♀; ventral surface with setation as in Fig. 13*d*.

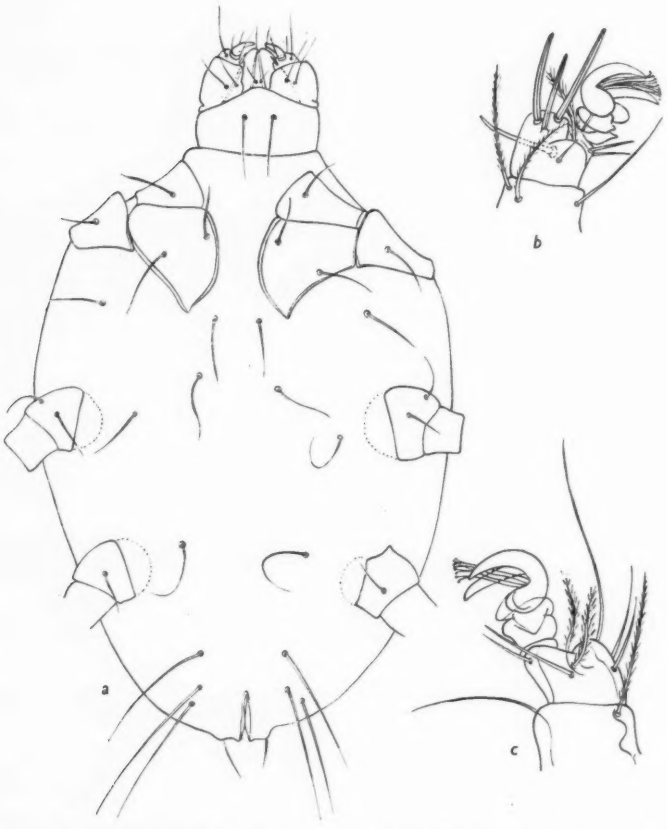


Fig. 12. *Ornithocheyla megaphallos* sp.nov. ♀: *a*, ventral surface (legs omitted); *b*, apex of leg I, dorsal; *c*, apex of leg II, postero-ventral view.

*Legs.* III and IV considerably longer than in the ♀, IV reaching far beyond posterior apex of abdomen; the posterior legs considerably more slender than the two anterior pairs, which are not relatively longer than in the ♀. Claws with the arrangement of ciliated hairs, sensory rods and empodium, as in the ♀.

*Dimensions.* Length of body 218$\mu$, greatest width 177$\mu$.

The genus is remarkable for the great size of the penis of the ♂. Male specimens seem to have been seldom found in the various genera of Cheyletidae, and often those specimens which differ in structure from the females do not appear to have

a penis. Baker in his monograph of the family (1949) does not figure a single male showing the penis, while Rodendorf (1940) gives only one such figure, a male of *Acaropsis docta* (*loc. cit.* fig. 26) in which the fairly large penis appears to be directed backwards. Vitzthum (1943), in a brief summing up, states that little is known of the ♂ genital system in Cheyletidae except that they possess a chitinous penis which emerges dorsally behind the boundary between the podosoma and opisthosoma.

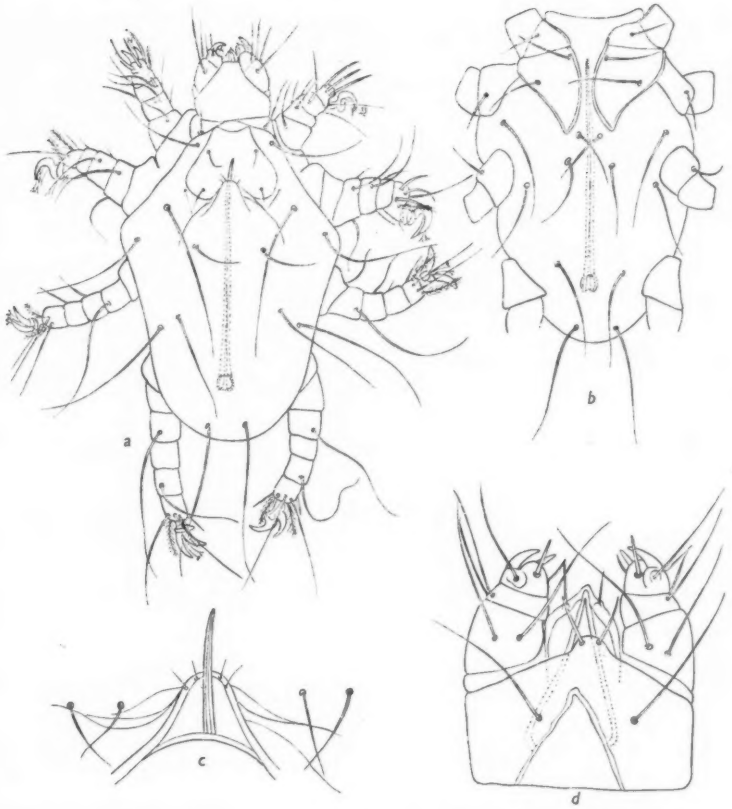


Fig. 13. *Ornithocheyla megaphallos* sp.nov. ♂: *a*, dorsal surface; *b*, ventral surface (appendages omitted); *c*, genital opening of dorsum enlarged; *d*, gnathosoma, ventral view.

The penis has its apex directed forwards and emerges more or less in the anterior half of the dorsal surface in the Myobiidae, and the following genera of Harpyrhynchidae and Cheyletidae: *Harpyrhynchus*, *Psorergates*, *Ophioptes*, *Ornithocheyla* and *Cheyletus* for certain, probably also in *Syringophilus* and *Picobia*, while *Acaropsis* may be and *Hemicheylus* is, an exception. In other related families of parasitic habit the penis is also directed forwards in *Demodex* and *Pterygosoma*. *Pterygosoma* however is an exception among the Pterygosomidae as other scale-

mite genera, such as *Gerrhosaurobia*, *Zonurobia*, *Geckobia*, *Hirstiella* and *Geckobiella* all have the penis directed backwards, emerging through the genito-anal opening at the extreme posterior apex of the body.

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# *METASTRONGYLUS APRI* THE PIG LUNGWORM

## OBSERVATIONS ON THE FREE-LIVING EMBRYONATED EGG AND THE LARVA IN THE INTERMEDIATE HOST

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(With 2 Figures in the Text)

The lungworms of the domestic pig belong to the genus *Metastrongylus*. Four species are known of which three, namely *M. apri* (*M. elongatus*), *M. pudendotectus* and *M. salmi* are said to occur in British pigs (Lapage, 1956). It is generally assumed that *M. apri* is the commonest of the three species, although there is only a limited amount of evidence to support this view. Lewis (1926) found both *M. apri* and *M. pudendotectus* in each of sixty pigs, but *M. apri* was present in greater numbers, while Robertson (1937) found *M. apri* to be more common than *M. pudendotectus* in 137 pigs infected with lungworms. The incidence of lungworm infection has recently been further investigated by Dunn, Gentles & White (1955). Unfortunately they did not attempt to identify the different species. They did, however, conclude that pig lungworms are more common than is generally realized.

During the past two years many infected pig lungs received from Croydon abattoir have been examined at Weybridge. *M. apri* was present in all of these lungs, often in large numbers, but *M. pudendotectus* was found in a few only, usually in small numbers, and *M. salmi* was never seen. *M. apri* was by far the commonest lungworm in the pigs sent to Croydon abattoir, so that all the available evidence seems to suggest that it is in fact the commonest species of pig lungworm in this country.

The life history of *M. apri* was elucidated by Hobmaier & Hobmaier in 1929. They demonstrated that the embryonated eggs voided in the pig's faeces were ingested by earthworms, and that development of the larvae to the infective stage took place in the hearts and within the anterior gut wall. The pig then became infected by ingesting the earthworm. They referred to the earthworms used in their experiments as *Lumbricus terrestris major* and *minor*. As these names have no zoological standing, it is not clear which species of earthworm they did in fact prove to be the intermediate host, but Schwartz & Alicata (1934), Shope (1941) and Dunn (1955) demonstrated subsequently that a number of species, including *Eisenia foetida*, *Allolobophora terrestris* f. *longa*, *A. terrestris* f. *typica*, *A. chloritica*, *A. caliginosa* f. *typica*, *Lumbricus terrestris* and *L. rubellus* can all serve as vectors.

Few other aspects of the life history have been investigated.

The viability of lungworm eggs was studied by Kates (1941) at Beltsville, U.S.A. He found that they survived from a maximum of 25 days, in faeces, on the surface of outdoor plots, to a maximum of 381 days in faeces buried in the soil. As he

worked with a mixture of *M. apri* and *M. pudendotectus*, it cannot be stated with certainty to what extent these figures do in fact apply to *M. apri*.

A number of investigators have expressed opinions, generally based on indirect observation, on the longevity of both the free-living, embryonated eggs and the larvae within the intermediate hosts, but these also refer to pig lungworms in general and not specifically to *M. apri*. It would seem that our knowledge of these phases of the life history is scanty. More detailed information is necessary if the transmission of this lungworm is to be understood, and if control measures are to be formulated on a sound basis. With this in mind preliminary studies on various aspects of the biology of the embryonated egg and the larva within the intermediate host have been carried out and are recorded in the present paper.

## MATERIALS AND METHODS

### *The embryonated egg*

*Observations out of doors.* A comparison was made between the viability of embryonated eggs in soil subjected solely to local climatic conditions and that of eggs in soil kept permanently wet. The ability of the eggs to infect the earthworm *Eisenia foetida* was used as an index of their viability. The first experiment was started in May 1956, but as the eggs in the non-watered soil died more rapidly than those in the watered soil, a second experiment using non-watered soil was set up in September 1956.

Embryonated eggs were obtained by mincing female worms extracted from pig lungs, and approximately 6000 eggs in 1 ml. of water were added to sterilized soil in each of a number of 4 in. plant pots. Some of these pots were placed in a shallow metal tray containing water to keep the soil permanently wet. The other pots were not watered in this way. The pots were kept out of doors, and every 4 weeks one pot from each group was taken into the laboratory and eight *E. foetida* from a stock culture were placed in the soil, which was kept moist to encourage the earthworms to remain active. After 3 weeks the earthworms were killed, and the anterior gut, cleaned of soil particles, together with the associated blood vessels, was examined microscopically for lungworm larvae, which were then separated from the earthworm's tissues and counted.

*Laboratory observations.* The effects of desiccation and of temperatures below freezing-point on the viability of the embryonated egg were investigated.

*The effects of desiccation.* Approximately 2000 embryonated eggs in 0·1 ml. of water were added to dry sterilized soil contained in each of a number of small glass tubes, which were kept at room temperature and humidity, until the soil was almost dry again. The tubes were then placed over sulphuric acid in desiccators in which the relative humidities were 50, 70, 80 and 95 %, respectively. Some tubes were left on the bench at a relative humidity of 65–82 %. Eggs were kept in moist soil (R.H. 100 %) to serve as controls. Each week the viability of the eggs was tested as already described, using four earthworms to each tube.

The effects of desiccation on eggs dried on slides and the effects of temperatures below freezing-point on eggs and first-stage larvae suspended in tap water, were

investigated using methods which have been described in detail elsewhere (Rose, 1955). The first-stage larvae were obtained by agitating a suspension of eggs in water.

### *The larva in the intermediate host*

Observations were made on the rate of development of the larva in the intermediate host at different temperatures, the longevity of the infective larva both within and when separated from the intermediate host, and the relationship between the length of time the larva remained in the earthworm and its subsequent infectivity.

#### *Infection of the intermediate host*

The earthworm *E. foetida* was used in all of the experiments. Attempts to infect individual worms by pipetting eggs into the mouth were not very successful. A more successful method was to keep the earthworms in a mixture of moist soil and embryonated eggs for 24 hr., after which they were thoroughly washed and transferred to clean moist soil.

#### *The rate of development at different temperatures*

The rate of development of the larvae was observed at 10–11°, 15–16° and 20–21° C.

As, at 10–11° C., the rate of development was very slow, an earthworm was killed and the larvae removed and examined microscopically at weekly intervals. At the higher temperatures development was more rapid and larvae were examined daily.

#### *Longevity and infectivity of the larvae in the intermediate host*

Larvae in earthworms which had been infected 2, 5, 7½, 9, 11 and 18 months previously were examined microscopically. As they were obviously alive, the infectivity of all but the oldest larvae, which were not available in sufficient numbers for testing, was assessed as follows: the anterior end of the earthworm was minced in physiological saline and the infective larvae thus liberated were collected by means of a pipette whilst being viewed under a dissecting microscope. A standard dose of 300 larvae in 0·5 ml. of saline was pipetted into the back of the throat of a guinea-pig and was followed by a further 0·5 ml. of saline. Five groups, each of three guinea-pigs, were dosed with larvae. After 3 weeks the guinea-pigs were autopsied and their lungs were examined. The bronchial tree was opened and any lungworms present were extracted and counted.

#### *Longevity of the infective larvae outside the intermediate host*

Larvae separated from the earthworm's tissues for use in the infectivity experiments were observed to survive for a considerable time when kept in the refrigerator. In view of this, a series of observations was made on the longevity of the infective larvae under different conditions.

Known numbers of infective larvae suspended in tap water were kept at temperatures of 5–6°, 10–11°, 15–16° C. and out of doors, where the temperature ranged

from 11 to 20° C. Larvae at 5–6° C. were examined microscopically at weekly intervals; those at the higher temperatures were examined more frequently and the numbers of living larvae were counted. Larvae were also subjected to temperatures below freezing-point, and to desiccation by being dried on slides, using the same methods as were employed with the embryonated eggs.

## RESULTS

The viability of the embryonated eggs out of doors in soil can be assessed from Fig. 1, which records the numbers of larvae present in the test earthworms. In the permanently moist soil eggs remained viable for a maximum of 2 years, whereas in the soil which was not watered artificially, longevity was less—5 months from May

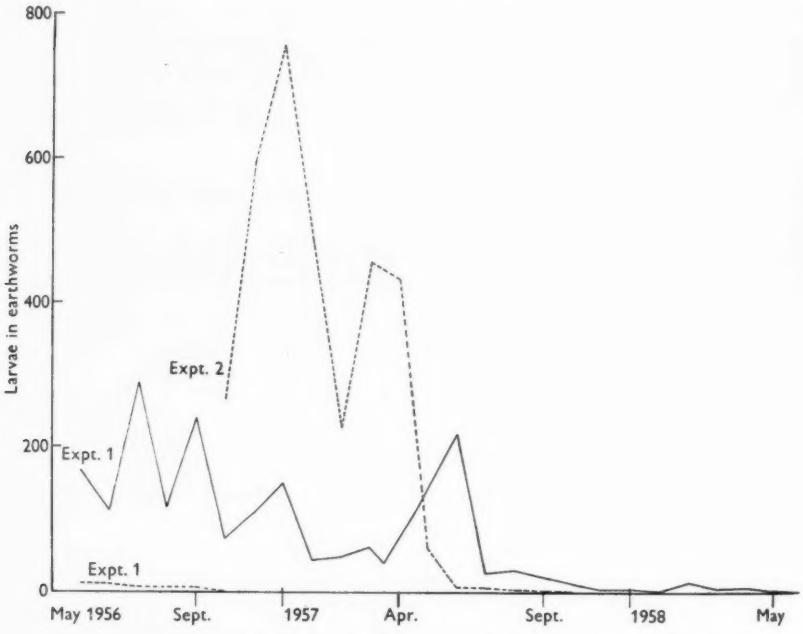


Fig. 1. The longevity of embryonated eggs in artificially watered and non-watered soil. —— watered soil; – – – non-watered soil.

to September 1956 (Expt. 1) and approximately 12 months from September 1956 to September 1957 (Expt. 2). This variation in the viability of the eggs appeared to be associated with variations in the moisture content of the soil, which in the case of the non-watered soil were related to the climatic conditions, in particular to the amount of rainfall. The rainfall in May 1956 was slight (Fig. 2), with the result that the non-watered soil was dry for most of the month—that is at the start of the experiment; and only thirteen larvae were present in the test earthworms examined in May. Subsequently fewer larvae were observed. In the second experiment with non-watered soil (started in September 1956) climatic conditions

were such that the soil remained moist more or less continuously until April 1957 when again there was little rainfall (Fig. 2) and the soil dried out and remained dry for some time. Many larvae were present in the test worms from this soil up to April, but then there was a sudden drop from 431 to 61. There was no comparable drop in the numbers of larvae in the test earthworms from the permanently watered soil, either in May 1956 or April 1957. As the only obvious difference was in the moisture content of the soil, it seems reasonable to assume that the embryonated eggs were unable to withstand desiccation for any length of time and had died as a result of the soil drying out. This assumption was confirmed by laboratory experiments. Eggs dried on slides survived for only a matter of days (Table 1).

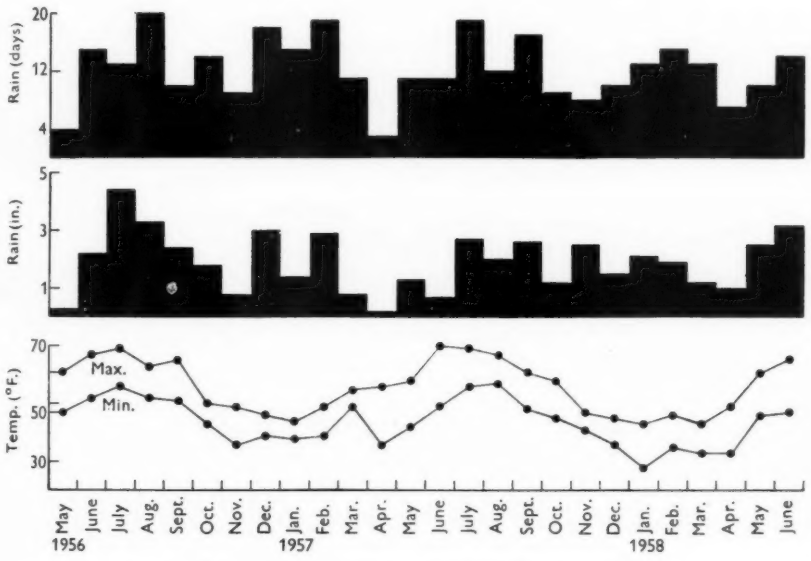


Fig. 2. Meteorological data, May 1956 to June 1958.

Table 1. *The effect of desiccation*

(The longevity of embryonated eggs when dried on glass slides.)

| | Percentage of living larvae | | | | |
|---|---|---|---|---|---|
| | Relative humidity | | | | |
| Days | 50% | 75% | 80% | 95% | Room humidity |
| 1 | 14 | 27 | 56 | 74 | 61 |
| 2 | 0 | 18 | 32 | 63 | 47 |
| 3 | — | 6 | 21 | 56 | 19 |
| 4 | — | 0 | 7 | 24 | 5 |
| 5 | — | — | 0 | 12 | 0 |
| 6 | — | — | — | 7 | — |
| 7 | — | — | — | 1 | — |
| 8 | — | — | — | 0 | — |

In dry soil longevity was greater, reaching a maximum of 20 weeks in an atmospheric relative humidity of 95%, but at lower humidities longevity was reduced (Table 2). In moist soil, however, larvae survived for many months.

Table 2. *The effect of desiccation*

(The longevity of embryonated eggs in dry soil.)

| | Larvae in earthworms | | | | |
|---|---|---|---|---|---|
| | Relative humidity | | | | |
| Weeks | 50% | 75% | 80% | 95% | Room humidity |
| 4 | 163 | 153 | 350 | 299 | 264 |
| 8 | 12 | 194 | 148 | 157 | 75 |
| 12 | 1 | 14 | 21 | 118 | 2 |
| 16 | 0 | 0 | 0 | 26 | 0 |
| 20 | 0 | 0 | 0 | 7 | 0 |
| 24 | — | — | — | 0 | — |
| 28 | — | — | — | 0 | — |

Table 3. *The longevity of eggs and first-stage larvae at temperatures below freezing-point*

| Weeks | Percentage of living eggs and larvae |
|---|---|
| 0 | 100 |
| 1 | 62 |
| 2 | 42 |
| 3 | 20 |
| 4 | 17 |
| 5 | 9 |
| 6 | 7 |
| 7 | 5 |
| 8 | 7 |
| 9 | 3 |
| 10 | 2 |
| 11 | 1 |
| 12 | 1 |
| 13 | 1 |
| 14 | 0 |
| 15 | 0 |

Eggs were able to overwinter despite a considerable amount of frost. Their ability to survive at temperatures below freezing-point was well illustrated in the laboratory experiment (Table 3), in which eggs and first-stage larvae survived for a maximum of 13 weeks at a temperature range of $-5$ to $-8°$ C.

Both eggs and first-stage larvae survived for many months at room temperatures.

The temperature did, however, have a marked effect on the rate of development of the larvae in the intermediate host. As the temperature was lowered, so the rate of development was reduced (Table 4).

By direct observation the larvae in the earthworms were seen to be still alive 18 months after infection. Larvae which had been in the intermediate host for 2, 5, 7½, 9 and 11 months were all capable of infecting guinea-pigs, as can be seen from Table 5, in which the numbers of adult worms recovered are tabulated.

Infective larvae, separated from the intermediate host and suspended in water, were able to survive for a surprisingly long time (Table 6). They even survived for a maximum of 2 weeks at temperatures below freezing-point (Table 6). When, however, they were dried on slides, they were all dead within 24 hr.

Table 4. *The rate of development of larvae in the intermediate host at different temperatures*

| | Minimum time in days to develop to | | |
|---|---|---|---|
| Temp. (°C.) | 2nd stage | 3rd granular | 3rd clear |
| 10–11 | 158 | 193 | 219 |
| 15–16 | 9 | 14 | 21 |
| 22–23 | 6 | 9 | 16 |

Table 5. *The effect of age on infectivity of the larvae*

| Time in earthworm (months) | No. of worms from guinea-pigs |
|---|---|
| 2 | 65 |
| 5 | 67 |
| 7½ | 90 |
| 9 | 42 |
| 11 | 25 |

Table 6. *The longevity of third-stage larvae freed from the intermediate host*

| Temp. (°C.) | Maximum longevity (weeks) |
|---|---|
| 5–6 | 62 |
| 10–11 | 16 |
| 15–16 | 7 |
| Out of doors 11–20 | 4 |
| −8–−5 | 2 |

## DISCUSSION

The life span of the embryonated egg in its natural environment is influenced by climatic factors and it appears that the most unfavourable of these is dryness. In the presence of moisture, however, it appears that other climatic factors are well tolerated and eggs in soil are capable of surviving up to 2 years or possibly longer.

The larvae within the intermediate host are protected to a great extent from changes in the external environment, although they are not completely immune. The rate of development within the earthworm is governed by the external temperature, so that under natural conditions it will vary at different times of the year. During the winter development will virtually cease.

The longevity of the larvae appears to be largely dependent on the longevity of the intermediate host, although the infective larvae can survive for some time when separated from the tissues of the earthworm. In the present observations the maximum observed longevity was 18 months, this being the longest period that

the earthworms survived after being infected. As the earthworms were adult when they were infected, their life span is clearly greater than 18 months. Recently, newly hatched earthworms have been infected, and it is anticipated that these will live for considerably longer, as no doubt the larvae also will. *E. foetida* has in fact been kept alive in captivity for 3½–4 years, while *Lumbricus terrestris* and *Allolobophora terrestris* f. *longa*, both of which can serve as the intermediate host, have survived for 5½–6 and 5½–10¼ years respectively (Cernosvitov & Evans, 1947). Unfortunately the longevity of earthworms under natural conditions is not known, but, in view of the hazards which beset them, it is likely to be less than in captivity. Even so, it is likely to be years rather than months.

It would seem, therefore, taking into consideration the longevity of both the free-living egg, and the larva within the intermediate host, that lungworm infestation on a pasture is capable of persisting for several years.

## SUMMARY

Observations out of doors demonstrated that the viability of embryonated eggs in soil was adversely affected by dryness. This was confirmed in laboratory experiments.

The resistance of embryonated eggs to temperatures below freezing-point was observed both out of doors and in the laboratory.

The rate of development of larvae in the intermediate host was reduced as the temperature fell.

The longevity of the infective larvae appeared to be largely determined by the longevity of the earthworm intermediate host, although they were able to survive for a time when separated from the earthworm's tissues.

The longevity of eggs and larvae under natural conditions and the persistence of infection on a pasture are discussed.

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# *RHIPICEPHALUS CAMELOPARDALIS* N.SP. (IXODOIDEA, IXODIDAE), A NEW SPECIES OF TICK FROM EAST AFRICAN GIRAFFES

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(With 4 Figures in the Text)

## INTRODUCTION

Among the ticks collected by Major E. W. Temple-Boreham from a giraffe at the Talek River, Cis-Mara area of W. Masai, Kenya, in October 1955, were eleven males and eight females which represent an undescribed species of the genus *Rhipicephalus*. Another female was collected by W. A. Hilton from a giraffe near mile 70 south of Arusha on the Great North Road, Tanganyika, on 10 July 1956, and G. H. Swynnerton collected three males from a giraffe at Nkonko, S. Manyoni district, Tanganyika, on 29 September 1958. The following is a description of these specimens.

### **Rhipicephalus camelopardalis** n.sp.

*Male* (Figs. 1–3)

*Salient features.* A large, inornate, reddish-brown tick. Capitulum large in relation to the size of the conscutum, basis capituli with lateral angles far forward and acute. Conscutum shiny, usually rather smooth and more or less rectangular in shape, body wall very distended, especially postero-laterally, in engorged specimens. Eyes relatively far back. Cervical pits present. Short marginal grooves sometimes present, sometimes merely represented by lines of punctations. Postero-median and postero-lateral grooves rather poorly defined. Shallow medium-sized punctations present, most noticeable on the shoulders, between the cervical pits and in lines along the marginal grooves, elsewhere scattered; very fine punctations scattered all over the conscutum. Numerous fine hairs on the ventral surface of the body, especially noticeable round the spiracles. Anal plates very broad posteriorly.

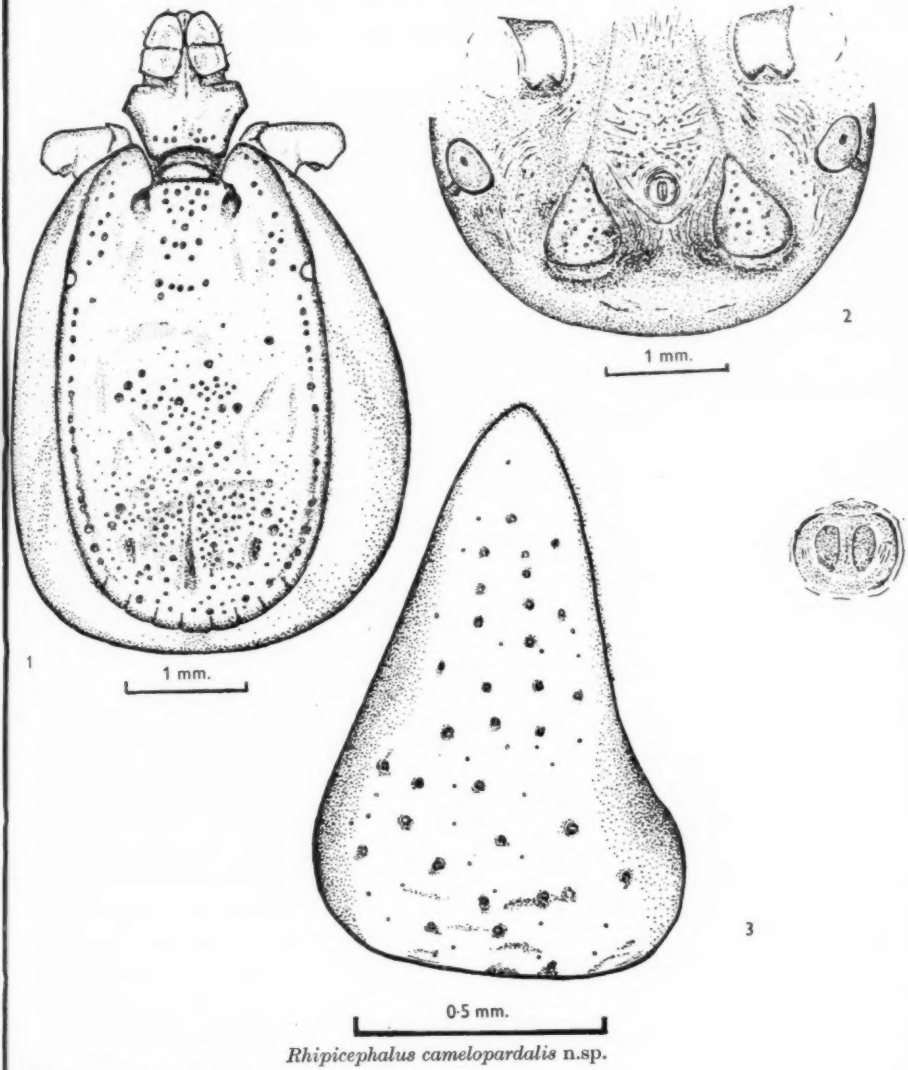
*Detailed description*

*Capitulum.* Slightly longer than broad, the length (including the cornua) × breadth varying from 1·5 × 1·2 to 1·3 × 1·1 mm. Subcollare present, oval in shape.

*Basis capituli.* Not quite twice as broad as long, the breadth × length (including the cornua) varying from 1·2 × 0·85 to 1·1 × 0·7 mm. Antero-lateral borders short, straight and divergent, postero-lateral borders long, concave and convergent.

The lateral angles are therefore acute and relatively anterior in position. Posterior border slightly concave with strong cornua. A number of small punctations are scattered across the basis capituli roughly parallel to the posterior border.

*Palps.* Article I easily visible from the dorsal surface. Articles II and III may be equal in length or article III may be slightly longer than article II. Both articles are broader than long.




*Rhipicephalus camelopardalis* n.sp.

Fig. 1. Male: dorsal view.

Fig. 2. Male: ventral view of posterior end, showing anal plates and spiracles. (The numerous hairs present on the ventral surface are not shown.)

Fig. 3. Male: anal plate.

*Conscutum.* Length × breadth varies from 4·9 × 3 to 4 × 2·5 mm. Inornate, reddish-brown in colour and not very heavily chitinized. Shiny and usually rather smooth in general appearance. It gives the impression of being almost rectangular in shape; the widest point is behind the eyes, then it tapers slightly posteriorly. All the specimens seen are engorged and in each the body wall is very much distended, especially postero-laterally, as shown in Fig. 1, but there is no caudal process. Anterior process of coxa I easily visible from dorsal surface. Emargination narrow and deep. Eyes nearly one-third of the way back, marginal, may bulge slightly, though their dorsal limits are flush with the conscutum, yellowish in colour. One or two punctations are sometimes, but not invariably, present adjacent to the eyes. Cervical pits deep, well marked. No cervical or lateral grooves but slightly depressed cervical fields present. Marginal grooves, when present, only extend forwards for a short distance from the posterior end of the conscutum. They are picked out by lines of punctations which continue forwards nearly to eye level. Sometimes there are no grooves at all, their positions merely being marked by punctations. Postero-median and postero-lateral grooves present, the former long and narrow, the latter short and broad. Festoons not well marked, with very shallow intervals.

Medium-sized and fine punctations are present, both types being shallow. Medium-sized punctations are grouped on the shoulders and between the cervical pits and form lines along the marginal grooves. Elsewhere they are scattered, except in the sunken cervical fields, where they are absent. Fine interstitial punctations are present all over the conscutum; anteriorly they may be exceedingly fine and sparse, especially in the cervical fields, posteriorly they usually become more numerous and more prominent and in some cases the area surrounding the postero-median and postero-lateral grooves is quite heavily punctate and almost rugose in appearance. Foveae cannot be distinguished.

*Legs.* Increase slightly in size from I to IV.

*Ventral surface.* Spiracle characteristic in shape, oval, with a short process projecting towards the dorsal surface. Numerous fine hairs are present on the ventral surface and are most pronounced on the body wall surrounding the spiracle, thus drawing attention to it.

*Anal plate* (Figs. 2, 3). Very broad posteriorly. External margin straight, joining the slightly convex posterior margin in a smooth curve. Internal margin scooped out opposite the anus, then becoming strongly convex and joining the posterior margin in a smooth curve. Numerous punctations present. In some particularly well-fed specimens the anal plates lift away from the ventral surface on protuberances of the body wall.

*Accessory anal plates* absent.

### *Female* (Fig. 4)

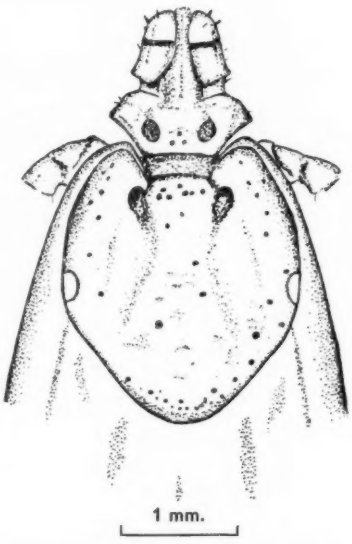
*Salient features.* A large, inornate tick. Capitulum large; lateral angles of the basis capituli far forward, the palps relatively long. Scutum slightly longer than broad with a sinuous posterior margin, shiny and smooth in general appearance. Eyes half-way back. Cervical pits and sunken cervical fields present. Shallow

medium-sized punctations present, more noticeable on the shoulders and between the cervical pits; very fine punctations scattered all over the scutum.

*Detailed description*

*Capitulum.* Usually very slightly longer than broad, the length × breadth varying from 1·4 × 1·35 to 1·2 × 1·15 mm. Subcollare present, broad and oval in shape, is sometimes difficult to see.

*Basis capituli.* Just over twice as broad as long, the breadth × length varying from 1·35 × 0·6 to 1·15 × 0·55 mm. Antero-lateral margins straight, divergent; postero-lateral margins concave, convergent; posterior margin straight; no cornua. Porose areas oval, slightly convergent, twice their diameter apart. A few punctations present between the porose areas.




*Rhipicephalus camelopardalis* n.sp.

Fig. 4. Female: dorsal view.

*Palps.* Article I long, easily visible from the dorsal surface. Articles II and III about the same length and each is about as broad as it is long.

*Scutum.* Slightly longer than broad, the length × breadth varying from 2·6 × 2·4 to 2·3 × 2·15 mm. Inornate, reddish-brown in colour, shiny and smooth. Broadest at eye-level, posterior margin sinuous. Emargination wide and quite deep. Eyes half-way back, slightly bulging, yellowish, one or two punctations may or may not be present adjacent to them. Cervical pits present, slightly convergent; no cervical or lateral grooves but sunken cervical fields present. As in the male, medium-sized and fine punctations present, both shallow. Medium punctations are grouped on the shoulders and between the cervical pits; elsewhere they are scattered, except

in the cervical fields, from which they are absent. Fine interstitial punctations are present all over the scutum but in most of the specimens examined they are so fine as to be almost invisible.

*Alloscutum.* Bears scattered fine white hairs which are, however, easily rubbed off.

*Ventral surface.* Spiracle resembles that of the male.

*Holotype.* Male collected from giraffe (*Giraffa camelopardalis tippelskirchi* Matschie) at the Talek River, Cis-Mara area of W. Masai, Kenya, in October 1955 by Major E. W. Temple-Boreham, deposited in the collection of the British Museum (Natural History), London.

*Allotype.* Female, same data as holotype, deposited in the collection of the British Museum (Natural History), London.

*Paratypes.* A single male and female, same data as holotype, are deposited in the collections of the British Museum (Natural History); the Rocky Mountain Laboratory, Hamilton, Montana, U.S.A.; the Veterinary Research Laboratory, Onderstepoort, South Africa; the Veterinary Research Station, Mpwapwa, Tanganyika, and in H. Hoogstraal's collection. Three males and two females, same data as holotype, are deposited in the collection at the Veterinary Research Laboratory, Kabete, and two males, same data as holotype, in the collection of the East African Veterinary Research Organization, Kikuyu. In addition one female, collected from a giraffe (*G. camelopardalis tippelskirchi*), near mile 70 south of Arusha on the Great North Road, Tanganyika, on 10 July 1956, by W. A. Hilton, and three males, collected from a giraffe (*G. camelopardalis tippelskirchi*), at Nkonko, S. Manyoni district, Tanganyika, on 29 September 1958 by G. H. Swynnerton, are in the collection of the East African Veterinary Research Organization.

## DISCUSSION

*Rhipicephalus camelopardalis* n.sp. is an easily recognized species and is one of the largest of the genus. In some respects it resembles *R. mühlensi*, although this is very much smaller. A comparison of the males of these two species shows, however, that the shape of their capituli is similar, both have a well-developed process on coxa I, there are similarities in the development of their dorsal grooves and the manner in which their body walls extend when they engorge is very much alike. In the females the general shape of the scuta of the two species is similar. Zumpt (1943) places *R. mühlensi* in the same group as *R. appendiculatus*.

*R. camelopardalis* may be a specific parasite of the giraffe, an animal from which comparatively few collections of ticks have been made in East Africa. If other hosts were commonly parasitized by this tick it seems probable that it would have been recorded before.

The nymph and larva are as yet unknown.

The authors wish to thank the Directors of the East African Veterinary Research Organization and the Kenya Veterinary Department for permission to publish this paper. They are also most grateful to Major E. W. Temple-Boreham, W. A.

Hilton and G. H. Swynnerton for collecting the ticks and to Dr G. Theiler and H. Hoogstraal for confirming that this is a new species, checking their manuscript and making numerous helpful suggestions.

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# DESCRIPTIONS OF TWO AMABILID CESTODES FROM THE LITTLE GREBE, *PODICEPS RUFICOLLIS*, WITH REMARKS ON THE FAMILY AMABILIIDAE BRAUN, 1900

By G. N. JOHRI

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(With 14 Figures in the Text)

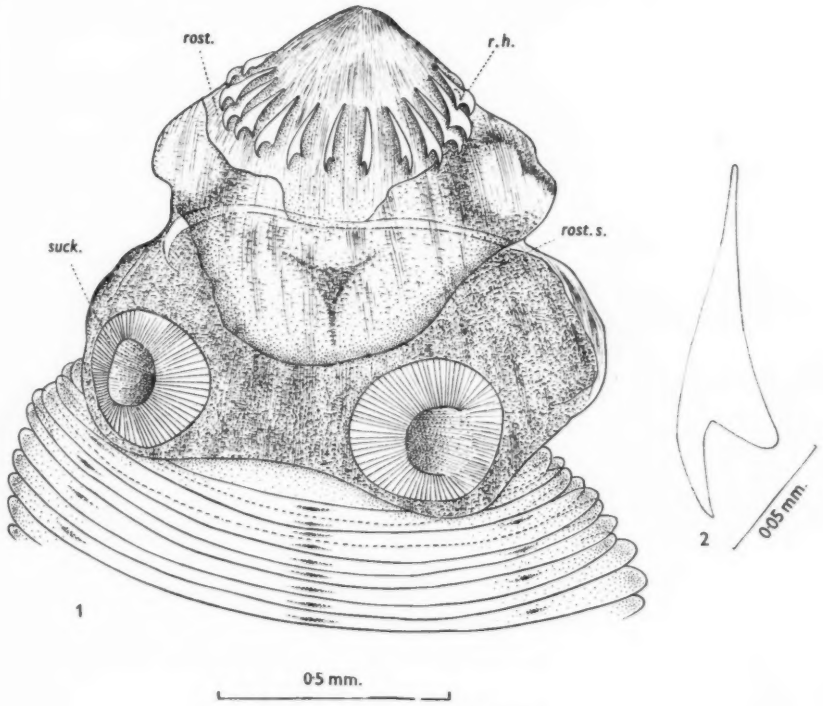
Of the two specimens of the little grebe, *Podiceps ruficollis* Vroeg shot in the environs of district Pilibhit, U.P., one harboured two mature specimens of the genus *Schistotaenia* Cohn, 1900 and a large number of specimens of the genus *Tatria* Kowalewski, 1904. Study of these specimens has shown that the former is a new species. So far as the author is aware, this is the first record of amabilid cestodes from India.

## **Schistotaenia indica** sp.nov.

The worms are 16–30 mm. long, with a maximum width of approximately 4–5 mm., including the lateral appendages. The scolex is large, powerful, highly muscular, conical, 1·00–1·07 mm. long and 1·13–1·46 mm. broad. It bears a strong rostellum with numerous layers of muscle fibres in it. The rostellum is 0·48–0·56 mm. long and 0·65–0·67 mm. wide, the rostellar sac being 0·62–0·80 mm. long and 0·67–0·85 mm. wide. Glandular cells at the base of the rostellum or minute spines all over it, as reported by Baer (1940) for *Schistotaenia scolopendra* Diesing, 1856, were not observed in these specimens. The suckers are spherical or oval in shape and measure 0·3–0·32mm. × 0·35 mm. and are provided with a very thick musculature. At the base of the dome-like rostellum are twenty hooks, arranged in a single row. They measure: from the proximal end of the base to the tip of the blade, 0·13–0·17 mm.; from the end of the base to the guard end. 0·09–0·12 mm., and from the guard end to the tip of the blade, 0·019–0·021 mm. A neck is absent, segmentation starting immediately behind the scolex.

The musculature of the strobila is rather weak. The most anterior immature segments are very narrow. The mature ones following them are 0·65–0·78 mm. long and 1·80–2·40 mm. wide, exclusive of the lateral appendages. The lateral appendages are very prominent and are directed backwards, more sharply in the posterior gravid segments, which are 0·60–0·80 mm. long and 1·96–2·54 mm. wide. The genital pores alternate irregularly and are located in the most anterior region of the lateral margin of the segments and very close to the anterior border of each segment. The genital cloaca is very inconspicuous and the vagina is absent. The cirrus-sac is very well developed in mature segments, in which it attains a length of about 0·37–0·40 mm. and a maximum width of 0·095 mm. The cirrus-sac passes between the dorsal and the ventral poral longitudinal excretory vessels. The wall of the cirrus-sac is muscular and thick and the cirrus is, in most segments,

everted, the everted portion measuring 0·40–0·50 mm. in length, with a maximum width of 0·06 mm. The cirrus is beset with spines and small hooks. An external vesicula seminalis is present, but is, in this species, only slightly differentiated; it is oval in shape and the vas deferens opens into it; it leads behind into a small glandular mass, the intermediate glandular pouch (i.g.p.), which in turn opens into the cirrus-sac. The glandular pouch is in young segments rather small, but it becomes distended with spermatozoa as the segments grow old. The glandular pouch and the seminal vesicle have a common covering of a muscular tissue and the cirrus-sac proper thus seems to be a single large sac with two structures




*Schistotaenia indica* sp.nov.

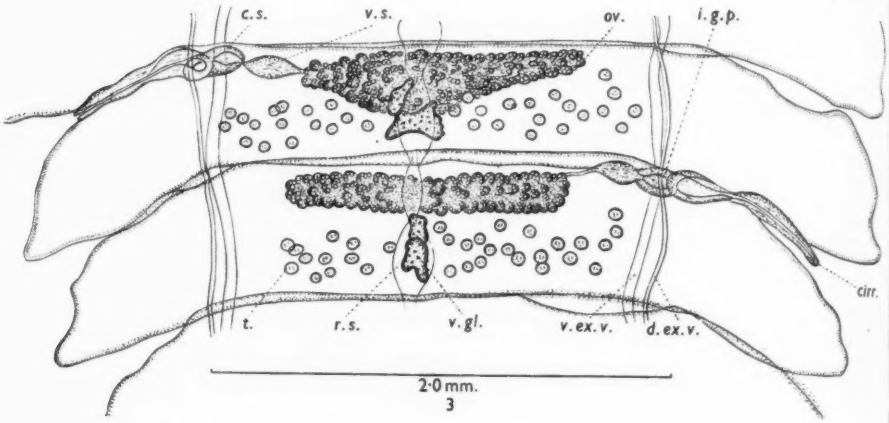
Fig. 1. Scolex. Fig. 2. Rostellar hook (enlarged).

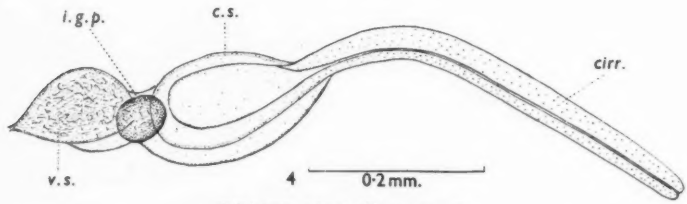
enclosed in it, but in young segments both these structures appear quite distinct and separate. Baer (1940) believes that both the seminal vesicle and the intermediate glandular pouch are modified portions of the vas deferens and the author, after a careful study of his specimens, is in full agreement with him.

There are about thirty to thirty-four testes in each segment, distributed almost equally on both the sides of the segment, occupying its posterior half behind the ovary, being limited within the longitudinal excretory vessels. They are spherical or oval and are 0·05–0·06 mm. in diameter. The ovary is densely lobulated, stretching across the anterior width of the segment within the longitudinal

excretory vessels. It measures 1·25 mm. across. The receptaculum seminis lies dorsally occupying a considerable portion in the middle of the segment and those of the successive segments communicate with each other. It attains a maximum diameter of 0·24 mm.

The vitelline gland is located posterior to the ovary and in certain cases it is notched in the median line. It measures 0·27–0·30 mm. across its widest part. The uterus begins as a lobulated sac, but later occupies the entire segment except



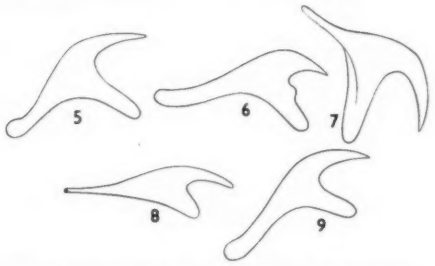

*Schistotaenia indica* sp.nov.

Fig. 3. Mature segment. Fig. 4. Cirrus-sac and associated parts (enlarged).

the lateral appendages. The eggs and the onchospheres are spherical, 0·052–0·056 mm. and 0·034–0·036 mm. in diameter, respectively. The embryonic hooks are not visible.

The genus *Schistotaenia* Cohn, 1900 includes only five species, *S. macrorhynchus* Rudolphi, 1810 (Krabbe, 1869; Cohn, 1900; and Clerc, 1907); *S. scolopendra* Diesing, 1856 (Baer, 1940); *S. macrocirrus* Chandler, 1948; *S. tennuicirrus* Chandler, 1948 and *S. colymba* Schell, 1955. The present form can be readily distinguished from all the above species by the size and the shape of its hooks and by the number of testes. It can be distinguished from *S. macrocirrus*, *S. tenuicirrus* and *S. colymba* by the number of its hooks and from the first two also by the size of the cirrus-sac which is, in the species here described, almost twice as large as it is in any of the known species excepting *S. macrocirrus* and *S. colymba*. It is further distinguished from *S. scolopendra*, *S. macrocirrus* and *S. colymba* by the length of its body, which is greater than that of the first

two of these three species and is much less than that of *S. colymba*. The absence of spines on the rostellum in the present form also separates it from *S. scolopendra*. It is, for these reasons, regarded as a new species and is named *Schistotaenia indica* sp.nov.




Rostellar hooks of known species of *Schistotaenia*

Fig. 5. *S. scolopendra*. Fig. 6. *S. macrorhyncha*. Fig. 7. *S. macrocirrus*. Fig. 8. *S. indica*. Fig. 9. *S. colymba*.

## Tatria acanthorhyncha Wedl, 1855

This species was described for the first time by Wedl (1855) from Europe. Mrazek (1927) studied its life cycle. Yamaguti (1940) redescribed it in detail from a Japanese grebe, *Podiceps ruficollis japonicus*. The present report of this species extends its range from Europe and Japan to India. In the following pages, an attempt has been made to describe this species in greater detail.

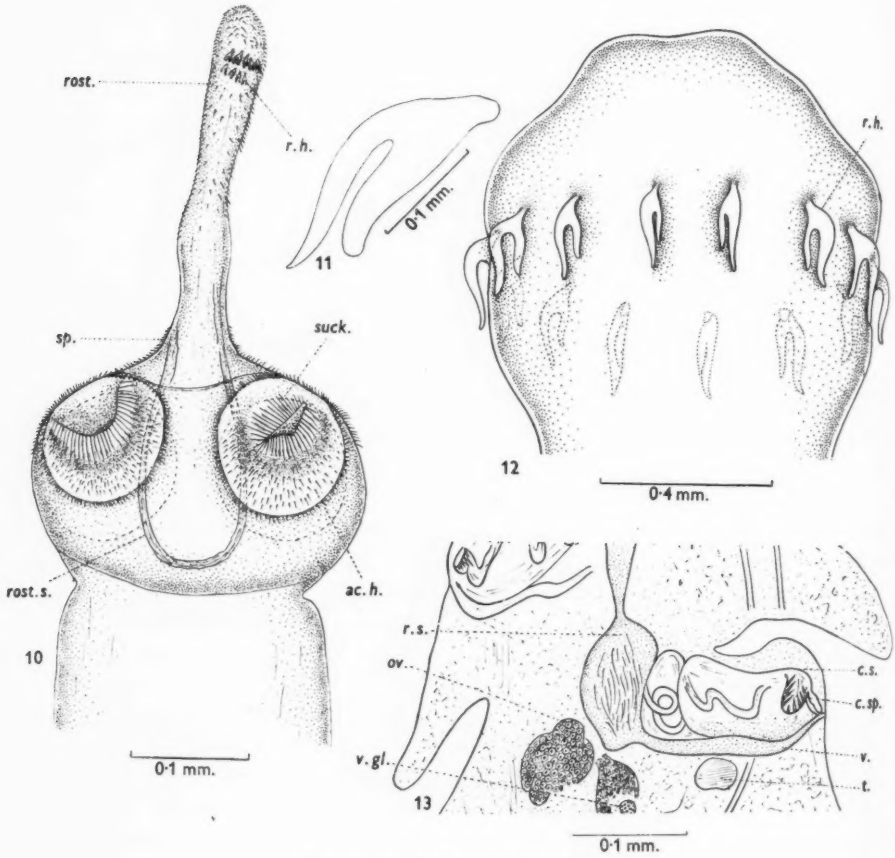
The worms are extremely small, 5–7 mm. long and 0·65 mm. wide. They have about 40–60 segments provided with lateral appendages which are directed posteriorly. The scolex is almost spherical, 0·21–0·23 mm. long and 0·29–0·35 mm. wide. Anteriorly the scolex bears an elongated rostellum 0·30–0·45 mm. long. The tip of the rostellum is slightly rounded, its diameter being 0·05 mm. and at the base about 0·06 mm. The rostellum bears a single row of fourteen rostellar hooks 0·015–0·020 mm. long. The handle of the rostellar hook is short and blunt, and 0·003–0·004 mm. long, while its guard and the blade are greatly elongated, measuring 0·009–0·012 mm. and 0·013–0·018 mm., respectively. Nearly half the length of the protruding part of the rostellum (distal region) and the anterior region of the scolex bear a large number of small accessory spines. A rostellar sac is very well developed, reaching almost to the base of the scolex, much below the lower level of the suckers, measuring 0·09 mm. in its maximum width.

The muscular suckers are rounded, 0·12–0·13 mm. in diameter and are provided with several rows of acetabular spines. A very short unsegmented region of the strobila is present behind the scolex and it measures 0·15 mm. in length and 0·32 mm. in width.

The genital pores alternate irregularly and are located on the lateral margin near the anterior border of the segments. A well-developed genital cloaca is present. The cirrus-sac is large, measuring 0·11–0·13 mm. in length with a maximum width of 0·04 mm. It passes between the dorsal and the ventral poral longitudinal excretory vessels and extends to a little less than half the width of the segment. The wall of the cirrus-sac is somewhat muscular and it encloses a coiled

cirrus densely beset with spines at its terminal portion. A small internal and a fairly extensive external vesicula seminales are present measuring 0·04 and 0·05 mm. in maximum diameter, respectively. The vas deferens is simple, straight and short.

The testes are large, but few, numbering five to eight in each segment. They occupy the whole width of the segment and are located mainly in the posterior




*Tatria acanthorhyncha* Wedl, 1855

Fig. 10. Scolex. Fig. 11. Rostellar hook (enlarged). Fig. 12. Anterior portion of rostellum (highly magnified). Fig. 13. Frontal section of a mature segment of *T. acanthorhyncha*.

half of thesegment and extend beyond the longitudinal excretory vessels. They measure 0·03 mm. in average diameter.

The ovary, 0·15–0·19 mm. × 0·04–0·06 mm. is median, distinctly bilobed and is located anterior to the testes. The two parts of the ovary extend on both the poral and the aporal sides of the segment. The vagina is a narrow tube just posterior to the cirrus-sac (also partly hidden by it). It has been possible to detect it only in sagittal sections (*v.* in Fig. 13).

The most interesting structure of the female genital organs is the receptaculum seminis. It communicates with its fellow in the next segment through a thin-walled duct. The vitelline gland is compact, situated behind the ovary and measures 0·07 mm. × 0·03 mm. In gravid segments the uterus appears as a transverse sac occupying the whole segment. Unfortunately, fully developed eggs were not present in the specimens examined.

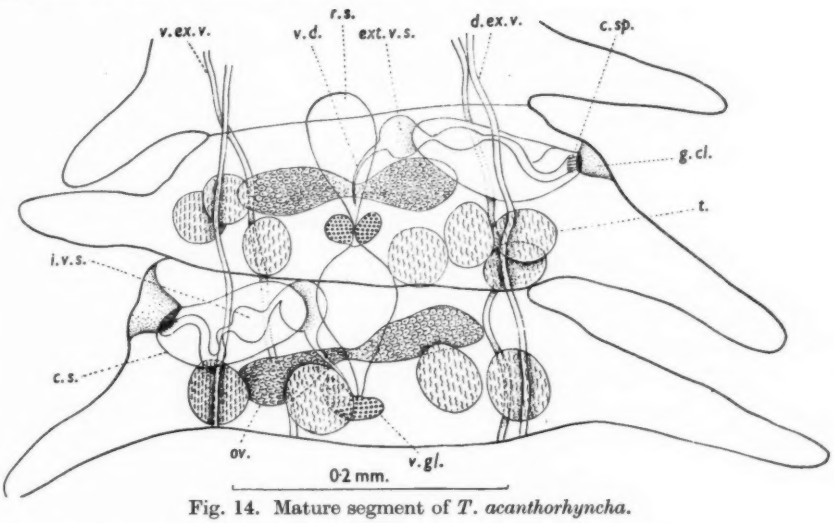



Fig. 14. Mature segment of *T. acanthorhyncha*.

## DISCUSSION OF THE SYSTEMATIC POSITION OF THE FAMILY AMABILIIDAE BRAUN, 1900

Rudolphi (1810) described a worm, *Taenia macrorhyncha*, which was later removed by Cohn (1900) to the new genus *Schistotaenia* created by him. Owen (1832) described a worm from the Old World flamingo, *Phoenicopterus ruber* and named it *Taenia lamelligera* on account of the prominent lateral extensions of its segments. Later on, Diamare (1893) removed the species *T. lamelligera* Owen, 1832 to his newly created genus *Amabilia* and named it *A. lamelligera*. Braun (1900) created the subfamily Amabiliinae of the family Taenidae for the reception of this species. Kowalewski (1904) created the genus *Tatria*, to which he also transferred *Taenia acanthorhyncha* Wedl, 1855. Fuhrmann (1907) raised the status of the subfamily Amabiliinae Braun, 1900 to that of a family, Amabilinidae, which was later correctly designated as Amabiliidae and placed three genera, *Amabilia* Diamare, 1893, *Schistotaenia* Cohn, 1900 and *Tatria* Kowalewski, 1904 in this family. The family was distinguished by the following characters.

Small forms with the scolex having an armed rostellum. Strobila provided with segments having prominent lateral extensions. Male genital pores single, regularly or irregularly alternating, or double and laterally situated. True vaginal pore and vagina absent, but in some individuals replaced in function by median accessory apertures situated dorsally and ventrally.

Although the creation of the family Amabiliidae, and the subsequent transfer

of *Amabilia, Schistotaenia* and *Tatria* to it, serves as a convenient grouping of these genera, there appears to be a wide gulf between the characters of *Amabilia* and those of *Schistotaenia* and *Tatria*. *Amabilia* Diamare, 1893 can be distinguished from *Schistotaenia* Cohn, 1900 and *Tatria* Kowalewski, 1904 by the presence of double male genital pores and double male genital organs with a single and median set of female genital organs. Furthermore, in this genus, the ovary and the vitelline gland are dendritic and the latter is much larger than the ovary.

The present investigation revealed that *Schistotaenia* and *Tatria* are more closely related and are very different from the genus *Amabilia*. *Schistotaenia* and *Tatria* are characterized by a single set of male and female genital organs and a typical receptaculum seminis, which is a thin-walled sac situated at the anterior border of the segments in the mid-line. The receptaculum seminis of each segment communicates with its fellow of the next segment through a small connexion, from which, or in some cases from near its vicinity, arises a narrow duct [Mrazek (1927), Baer (1940) and Chandler (1948)] functioning as a vagina. The close affinity and relationship of these two genera is further strengthened by their host specificity, being exclusively parasites of grebes. *Amabilia*, possessing totally different types of character as already enumerated, shows great contrast and stands apart from the other two genera. It is therefore proposed that the family Amabiliidae Braun, 1900 should be split up into the two subfamilies, Amabiliinae Braun, 1900 for the reception of the genus *Amabilia* Diamare, 1893 and Schistotiinae subfam.nov. for the genera *Schistotaenia* Cohn, 1900 and *Tatria* Kowalewski, 1904.

## Family AMABILIIDAE Braun, 1900

*Key to the subfamilies proposed*

| | |
|---|---|
| Male genital organs and genital pores double in each segment | *Amabiliinae* Braun, 1900 |
| Male genital organs and genital pores single in each segment | *Schistotiinae* subfam.nov. |

### AMABILIINAE Braun, 1900

Of the family Amabiliidae. Cestodes of small size. Rostellum armed. Male genital organs double in each segment with double genital pores. Cirrus armed with strong spines. Female genital organs single in each segment and median in position. Ovary much smaller than the vitelline gland, both being dendritic. Vagina opening into a dorso-ventral osmoregulatory canal, which opens both dorsally and ventrally. Adults in the flamingo.

Type genus **Amabilia** Diamare, 1893

### SCHISTOTIINAE subfam. nov.

Of the family Amabiliidae. Cestodes of small size. Rostellum armed and powerful. Male genital organs single in each segment. Genital pores single, alternating regularly or irregularly. Cirrus spiny or non-spiny. Female genital organs single in each segment and median in position. Ovary larger than the vitelline gland and bilobed. True vagina absent, replaced in function by a short duct arising from the receptaculum seminis. Adults in grebes.

Type genus **Schistotaenia** Cohn, 1900

The author is highly indebted to Professor M. B. Lal for his guidance in carrying out the present work and also to Dr L. N. Johri of Delhi University for his keen interest and for reading the manuscript of this paper.

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## ABBREVIATIONS USED

| | | | |
|---|---|---|---|
| *ac.h.* | acetabular hook | *rost.* | rostellum |
| *c.s.* | cirrus-sac | *rost.s.* | rostellar sac |
| *c.sp.* | cirrus spine | *sp.* | spine |
| *cirr.* | cirrus | *suck.* | sucker |
| *d.ex.v.* | dorsal longitudinal excretory vessel | *t.* | testis |
| *ext.v.s.* | external vesicula seminalis | *v.* | vagina |
| *g.cl.* | genital cloaca | *v.d.* | vas deferens |
| *i.g.p.* | intermediate glandular pouch | *v.ex.v.* | ventral longitudinal excretory vessel |
| *i.v.s.* | internal vesicula seminalis | *v.s.* | vesicula seminalis |
| *ov.* | ovary | *v.gl.* | vitelline gland |
| *r.h.* | rostellar hook | | |
| *r.s.* | rostellar sac | | |

(*MS. received for publication* 11. xii. 1958.—Ed.)

# ADDITIONAL HOST AND LOCALITY RECORDS FOR *GECKOBIELLA TEXANA* (BANKS), 1904 (ACARINA, PTERYGOSOMIDAE)

By K. M. JACK

*2 Derwent Close, Gossops Green, Crawley, Sussex*

The New World iguanid lizards of the genus *Sceloporus* are parasitized by the scale-mite *Geckobiella texana* (Banks) 1904. This species has been sufficiently described by Banks (1904, 1906, 1907, 1915), Hirst (1917, 1926), Lawrence (1953) and Lane (1954), while the life history has been worked out by Goodwin (1954).

The host and locality records for this scale-mite so far published are:

| Host | Locality | Source |
|---|---|---|
| *Sceloporus undulatus undulatus* | Georgia | Lane, 1954 |
| *S. undulatus* | Durham, N.C. | Lawrence, 1953 |
| *S. floridanus* | Travis Co., Texas | Banks, 1904 |
| *S. spinosus clarkii* | Duval Co., Texas | Hirst, 1917 |

This species has been found to occur on the following species of *Sceloporus* in the collections of the British Museum (Nat. Hist.). I would like to take this opportunity of expressing my thanks to the Museum Authorities and to the Staff of the Reptile Section for so readily making available the collections and facilities for study.

| Host | Locality |
|---|---|
| *S. torquatus* | Tapotlan |
| | Xarhimilco, near Mexico City |
| *S. torquatus poinsettii* | Ciudad, Durango |
| *S. torquatus melanogaster* | El Paso, Texas |
| *S. spinosus* | Puebla |
| | Tehuantepec |
| *S. spinosus clarkii* | Fort Lowell, Arizona |
| *S. spinosus horridus* | Chilpancingo, Guerrero, Mexico |
| | La Cumba les Arrastrados, Jalisco |
| | Orilla, Michoacau |
| *S. undulatus* | Hot Springs, Arkansas |
| | Cadd's Parrish, Louisiana |
| *S. undulatus occidentalis* | Hot Springs, Arkansas |
| *S. undulatus bocourti* | Marin Co., California |

| Host | Locality |
|---|---|
| *S. orcutti* | San Jacinto, California |
| | San Diego Co., California |
| *S. pyrrocephalus* | Colima, Mexico |
| | Manzanillo, Mexico |
| | Joculla, Michoacau |
| *S. cupreus cupreus* | Sarabia, Atlantic Slope |
| | Santo Domingo del Guzman, Atlantic Slope |
| *S. siniferus* | Tehuantepec City |
| | Salina Cruz, Tehuantepec |
| *S. acanthinus* | Bebedero, Costa Rica |
| | Oaxaca |
| *S. yarrowii* | Boioina Sierra, Jahaeumaec |
| *S. ornatus* | Meadow Valley, Chihuahua, Mexico |
| *S. formosus* | Duenas, Guatemala |
| *S. variabilis* | Agua Fina, Vera Cruz |
| *S. utiformis* | Tepic, Mexico |

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# THE MORPHOLOGY AND LIFE CYCLE OF *PLISTOPHORA SCATOPSI* SP.NOV., A MICROSPORIDIAN PARASITIC IN THE MID-GUT OF *SCATOPSE NOTATA* MG. (DIPTERA, NEMATOCERA)

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(With 53 Figures in the Text)

## INTRODUCTION

Although microsporidia have been reported in many dipterous larvae, there is only one mention of their occurrence in Scatopsids. Keilin (1919) noted that Scatopsid larvae were infected with a microsporidian parasite 'probably a *Glugea*, which invades the epithelial cells of the mid-gut and caeca'. He does not mention the species of the host. The present paper describes a microsporidian which occurs in the mid-gut and caeca in the larvae of *Scatopse notata* Mg. It seems likely that it is the same parasite as that observed by Keilin. The infected larvae were found in potatoes which had been left to rot in the open. The author's attention was first drawn to the parasite by Dr P. Tate, who observed the parasitic spores while dissecting the larvae of *S. notata*.

## MATERIALS AND METHODS

The infected larvae were kept in continuous mass culture in the laboratory on fresh and rotting potato. Smaller cultures were raised in glass containers on 5% yeast agar (1·5% agar-agar containing 5% whole yeast). This medium was found to be perfectly satisfactory for rearing the host larvae, and they passed through their life cycle normally. If batches of eggs were isolated when laid, the larvae which hatched from them could be kept uninfected throughout their life cycle by rearing them on this artificial medium. Such 'clean' larvae could be infected by feeding them with agar which had been smeared either with a suspension of spores in saline obtained from infected larvae, or with the infected guts. The life-history of the parasite was followed from smears of the larval guts and sections made daily from such artificially infected larvae. Smears were either dry-fixed and stained with Giemsa's stain, or wet-fixed with Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. Kohn's (1957) stain, which requires no prior fixative, was also used for wet smears. Whole larvae for sectioning were fixed in Carnoy's fixative before being stained with haemalum and eosin, iron haematoxylin, or by the Giemsa–Colophium method (Shortt & Cooper, 1948). To ensure rapid penetration of the fixative the posterior end of the larvae was cut off before it was fixed. Alternatively, the guts were dissected out and fixed.

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### *Occurrence of the parasite*

The incidence of naturally occurring infection is extremely high, and although no figures are available it is thought that the incidence may be as high as 100%. Young larvae reared under 'clean' conditions quickly become parasitized when they are placed with infected larvae. Externally, parasitized larvae appear normal, although in the fourth instar they sometimes acquire a whitish appearance. This may be due to the normal increase of fat body prior to pupation. In infected guts, examined under low-power magnification, the mid-gut and caeca present a mottled appearance, dark, round areas being clearly visible against the lighter normal tissues. Under higher magnification, these masses are seen to be large aggregations of spores and earlier stages of the parasite. These areas closely resemble cysts and no doubt it was this feature which led Keilin to suggest that the parasite belonged to the genus *Glugea*. However, the masses in this instance are not cysts but host cells enclosing such a large number of spores that little, if any, of the host cytoplasm is visible. In addition to these aggregations of spores, single spores can be seen scattered throughout the cells of the mid-gut and caeca. The parasite has never been observed in tissues of the larvae other than those of the mid-gut. Spores do, however, occur in both the pupae and the adult flies. This suggests that infective spores may reach the exterior and a new host only after the death of the host fly, or when the remains of the larval gut, which are enclosed in the adult gut, and in which the spores occur, are voided. Spores, or any other stage of the parasite, have never been observed in the rudiments of the gonads which are present in the fourth larval instar, or in the eggs or smears of eggs laid by infected flies. Furthermore, larvae reared on agar under 'clean' conditions from such eggs do not develop the infection. It is concluded that the parasite does not pass through the egg of the host to the next generation as occurs with some microsporidian infections, e.g. in the silkworm hosts of *Nosema bombycis*. The occasional occurrence of single spores in the lumen of the gut suggests that spores may reach the exterior by this route after the rupture of the infected cells. Such a mode of transmission is described by Chatton (1907) in *Plistophora intestinalis*, but in Scatopsid larvae spores are seen so infrequently in the lumen of the gut that probably their presence there results accidentally during dissection and it is not their normal route to the exterior.

The related species of *Scatopse*, *S. fuscipes*, occurs in close contact with *S. notata* under natural conditions. Although the larvae of both species intermingle freely and have been reared in close contact with one another in the laboratory on infected media, the parasite has never been observed in the tissues of *S. fuscipes*.

### *Morphology and development*

#### (*a*) *Schizogony*

The earliest stages observed in smears are round uninucleate bodies about 3$\mu$ in diameter, with dense cytoplasm which stains deeply with Giemsa's stain (Fig. 1). These forms, which are presumably schizonts, appear in smears of the guts together with some later stages, 9–14 days after the larvae have been artificially

infected. Presumably this is the time needed for passage of the spore down the larval gut, the extrusion of the filament, and the entry of the infective sporoplasm into the host cell. It is not known how the sporoplasm enters the host cell. Once within the host cell schizogony proceeds by binary or multiple fission (Figs. 4, 6), nuclear division not always being followed by cytoplasmic cleavage. Multinucleate schizonts with up to sixteen nuclei may thus be formed (Figs. 7–10). It is possible that schizonts with more than sixteen nuclei are formed, but it is difficult to be certain of this as such forms would closely resemble the multinucleate sporonts which are formed later. The products of fission may re-enter the cycle. No secondary schizogony such as is reported in *Nosema helminthorum* by Dissanaike (1958) has been observed.

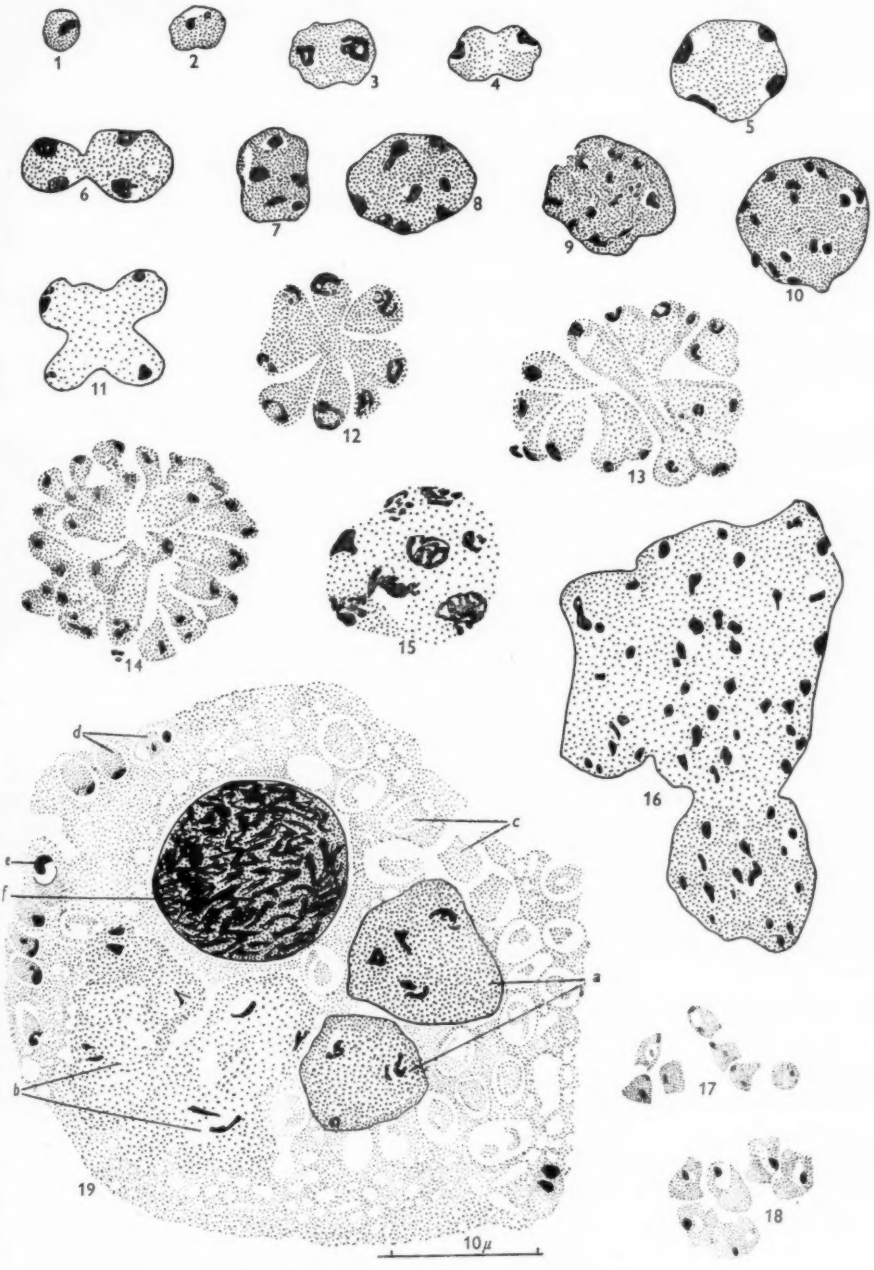
(*b*) *Sporogony*

The onset of sporogony is marked by the formation of sporonts. The exact point in the life cycle at which this takes place has not been determined. It is more probable that the multinucleate schizonts transform directly into sporonts than that the uninucleate schizonts formed by fission transform into uninucleate sporonts. This question of when schizogony ends and sporogony commences is further considered below. The later sporonts may be distinguished from schizonts by their more diffuse cytoplasm, which stains less deeply with Giemsa's stain, their irregular outline, and sometimes by a clear area around the nuclei (Fig. 15). The nuclei are often vesicular rather than compact as are the schizont nuclei. Multinucleate sporonts with more than sixteen nuclei are frequently observed, and sometimes bizarre forms with over fifty nuclei are seen (Fig. 16). Sporoblasts are formed from the sporonts in two different ways. It has not been determined whether this results in the formation of two kinds of spores.

(i) The cytoplasm of the sporont becomes even more diffuse and the nucleus vesicular. The definition of the parasite may become lost against the background of the host cytoplasm (Fig. 19). The sporonts finally break up into sporoblasts, each nucleus and a portion of the sporont cytoplasm forming a sporoblast. There is no constriction of the cytoplasm, merely a 'parcelling up' of the sporont cytoplasm. Each uninucleate sporoblast so formed eventually gives rise to one

Legends to Figs. 1–19

Figs. 1–19. Developmental stages of *Plistophora scatopsi* sp.nov. in gut smears of the larvae of *Scatopse notata*. Stained with Giemsa's stain. Scale as for Fig. 19. Figs. 1–10. Schizogony. Fig. 1. Uninucleate schizont. Fig. 2. Binucleate schizont. Fig. 3. Binucleate schizont, nuclei about to divide. Fig. 4. Binucleate schizont undergoing fission. Fig. 5. Tetranucleate schizont. Fig. 6. Tetranucleate schizont undergoing fission. Figs. 7–10. Multinucleate schizonts. Figs. 11–19. Sporogony. Figs. 11–14. Pansporoblasts of varying numbers of sporoblasts. Fig. 15. Sporont, prior to formation of sporoblasts by breaking up of sporont. Fig. 16. Sporont with over fifty nuclei. Fig. 17. Young sporoblasts, some with developing polar cap. Fig. 18. Young sporoblasts. Fig. 19. Host cell enclosing stages of sporogony. *a*, young sporonts, outline still clear; *b*, late sporonts, outline lost against background of host cytoplasm; *c*, young spores, membrane just formed; *d*, spores, polar cap and membrane formed; *e*, spore showing crescent shape of polar cap; *f*, host cell nucleus.




Figs. 1–19. For legends see opposite page

spore. In an area of host tissue, or even in a single host cell, all the stages of sporogony may be seen (Fig. 19).

(ii) The multinucleate sporonts may give rise to pansporoblasts. The sporont nuclei migrate to the periphery and the cytoplasm constricts in towards the centre. Thus a pansporoblast is formed, which is composed of many sporoblasts still in continuation with one another at the centre. Pansporoblasts of varying numbers of sporoblasts have been observed (Figs. 11–14). Each sporoblast gives rise to a single spore, sometimes first breaking away from the main body of the pansporoblast. At other times the spore is formed within the pansporoblast although the connexion at the centre is severed.

The formation of the spore is best followed when it is formed by the direct breakdown of the sporont. Sporoblasts formed in this manner are irregularly shaped, uninucleate bodies, the cytoplasm of which stains very lightly with Giemsa's stain (Figs. 17, 18). The spore membrane is then developed, its presence being indicated by a clear, oval-shaped area formed round the sporoblast (Figs. 19, 25, 29). A round body, the polar cap, is also formed at this stage, first appearing as a small body in the cytoplasm of the sporoblast (Figs. 30, 31), and gradually enlarging until it forms a heavy cap at the 'anterior' end of the spore (Figs. 26, 27, 29, 32). The polar cap becomes less prominent again in mature spores such as are found in host flies (Fig. 36). Sometimes the polar cap is developed before the spore membrane (Fig. 17), sometimes the latter is first formed (Fig. 25). The cytoplasm of the sporoblast then takes up a more central position in the young spore, forming the uninucleate sporoplasm which separates the 'anterior' end with its polar cap from the vacuole which is formed in the 'posterior' end of the spore.

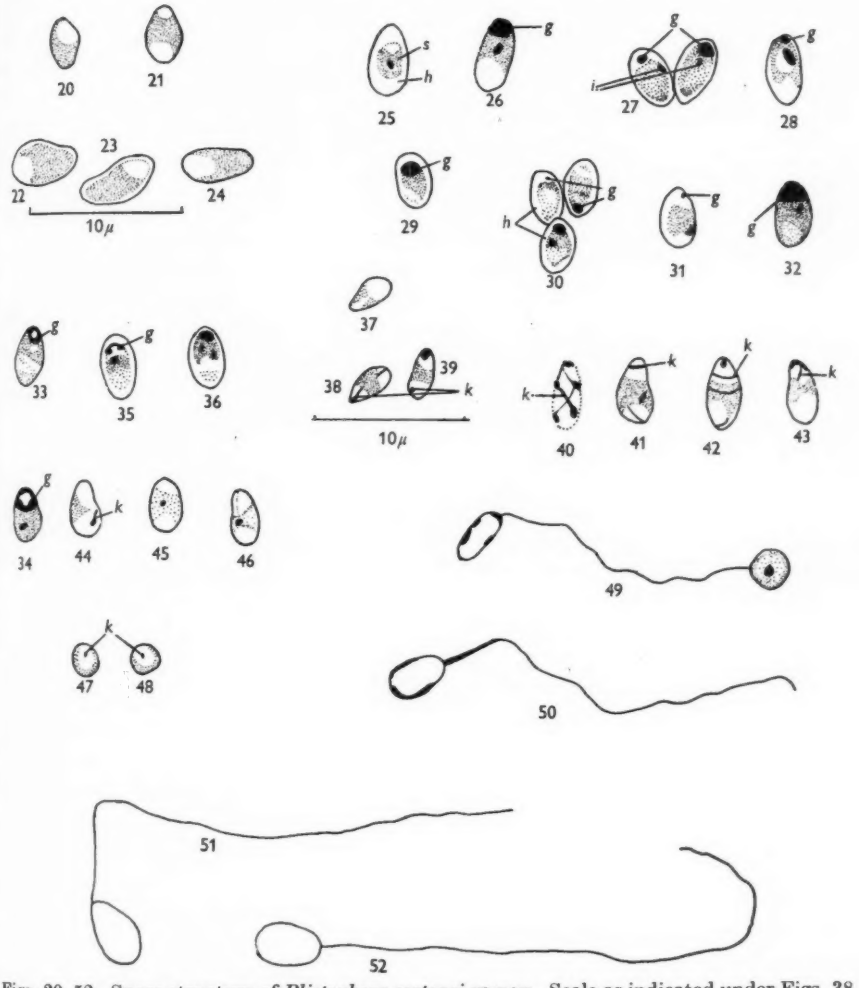
(c) *Structure of the spore*

The mature spore, when observed in fresh preparations in saline, is a pear-shaped body 4–6$\mu$ long and 2–3$\mu$ in diameter. The transparent membrane is indicated by a refractive area surrounding the spore (Fig. 22). The posterior vacuole is clearly seen, as is also the central band of the sporoplasm (Figs. 20, 21). In stained smears and sections the sporoplasm is seen to be a crescent-shaped girdle surrounding the centre of the spore immediately beneath the membrane (Figs. 37, 44, 47, 48). In the majority of preparations only one nucleus is visible in the sporoplasm, but in some two nuclei are seen (Fig. 36). However, the rare occurrence of two nuclei in preparations, and the fact that when ejected the sporoplasm is uninucleate, suggest that the apparent second nucleus is an artifact, or possibly a section of the coiled polar filament.

That the polar cap is not composed of nuclear material is demonstrated in wet smears stained with Kohn's stain. This is primarily a chromatin stain, and although the sporoplasm nucleus is clearly shown in such preparations, the polar cap does not take up the stain (Figs. 44–46). In some smears it is clearly shown that the polar cap is a circular or crescent-shaped body (Figs. 33–35).

In smears stained with Kohn's stain or with iron haematoxylin and over-differentiated, the polar filament is visible within the spore. In some spores the filament appears to arise from the polar cap (Figs. 38, 43). The filament is coiled

within the whole of the interior of the spore and there is no indication of a polar capsule. The filament lies beneath the sporoplasm and is only visible in this area if the sporoplasm is not too deeply stained (Figs. 38, 40–42). In some preparations




Figs. 20–52. Spore structure of *Plistophora scatopsi* sp.nov. Scale as indicated under Figs. 38 and 39 for all figures except Figs. 20–24. Figs. 20–24. Fresh spores in saline. Figs. 25–32. Young spores in smears stained with Giemsa's stain, showing development of membrane and polar cap. Figs. 33–35. Spores in smears stained with Giemsa's stain, showing structure of polar cap. Fig. 36. Mature spore from fly. Giemsa's stain. Fig. 37. Spore showing girdle of sporoplasm. Heidenhain's haematoxylin. Figs. 38–43. Spores stained with Heidenhain's haematoxylin showing filament within the spore. Figs. 44–46. Spores stained with Kohn's stain. Figs. 47, 48. Spores in section to show girdle of sporoplasm. Stained with haemalum and eosin. Fig. 49. Empty spore with extruded filament and sporoplasm. Smear preparation stained with Giemsa's stain. Fig. 50. Empty spore with extruded filament. Giemsa's stain. Figs. 51, 52. Spores in saline with extruded filaments. *g*, polar cap; *h*, spore membrane; *i*, nucleus; *k*, polar filament; *s*, sporoblast.

a small dot is seen in the posterior vacuole. This is not a nuclear body, or a metachromatic granule such as is described by Dissanaike (1957) in *N. helminthorum*, but is the filament seen in section or 'end on'. This is clearly demonstrated if such preparations are examined in different planes under the microscope (Figs. 39, 44).

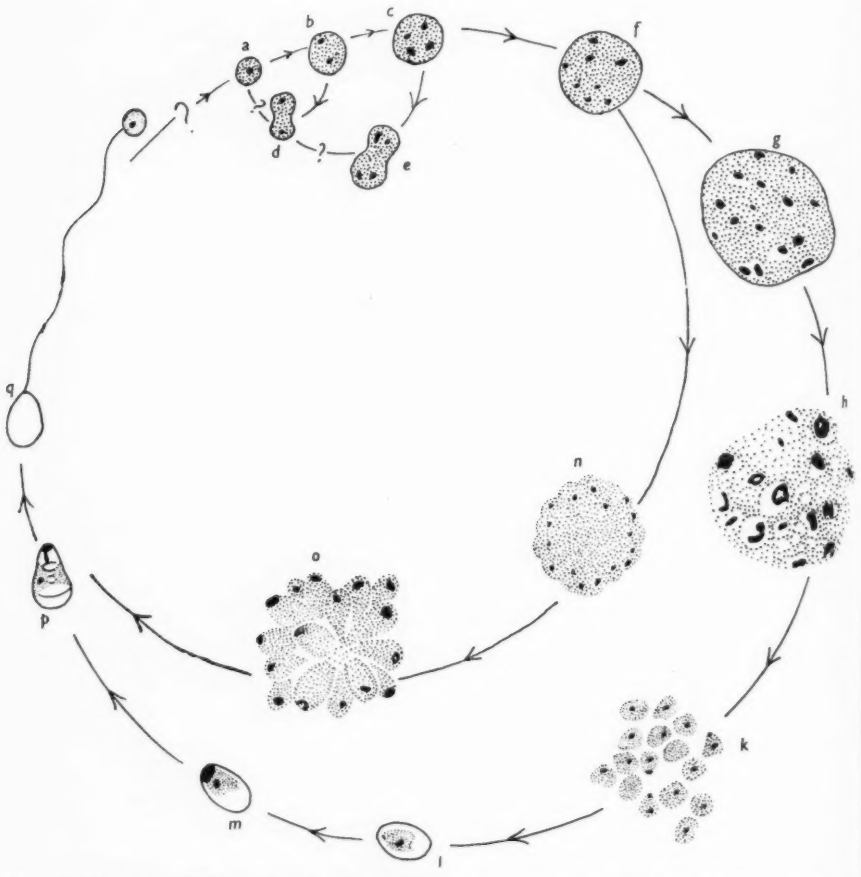



Fig. 53. Diagram of life cycle of *Plistophora scatopsi* sp.nov. Not to scale. Schizogony: *a*, uninucleate schizont; *b*, *c*, *f*, multinucleate schizonts; *d*, binucleate schizont undergoing binary fission; *e*, tetranucleate schizont undergoing fission; *g*, early sporont; *h*, late sporont showing irregular outline, diffuse cytoplasm, vesicular nuclei, immediately prior to breakdown; *k*, sporoblasts formed by breaking up of sporont; *l*, young spore with membrane and polar cap forming; *m*, spore with prominent polar cap; *n*, sporont prior to formation of pansporoblast; *o*, pansporoblast; *p*, mature spore; *q*, infective spore with extruded filament and sporoplasm.

Under certain conditions, which will not be elaborated here, the filament may be extruded. In fresh preparations the filament measures 30–40$\mu$ in length. In some fresh preparations the spherical, uninucleate sporoplasm may be seen at the tip of the extruded filament. The mechanism of the escape of the sporoplasm from

the spore will not be discussed, but since the spore appears to be empty in both stained and fresh preparations (Figs. 49–52), the mass at the tip of the extruded filament clearly represents the sporoplasm of the mature spore.

## DISCUSSION

It is necessary to consider the point at which sporogony commences. It might be argued that the multinucleate bodies, which are termed sporonts above, are in reality multinucleate schizonts which break up to form uninucleate sporonts each of which later gives rise to a single spore. However, the alternative formation of spores from pansporoblasts makes such a course of development unlikely. The pansporoblasts are formed directly from multinucleate bodies and it is therefore considered that the large, irregular, multinucleate bodies are sporonts, as described, and not schizonts. Similar forms are described in a microsporidian, *Plistophora collessi*, found in certain mosquitoes by Laird (1959), and in another *Plistophora* described by Bosanquet (1910). Bosanquet considers that the multinucleate, irregular bodies which he observed were sporonts, and that following their formation 'it would seem as if the whole body of the sporont had broken up as a preliminary to spore formation'. It is therefore concluded that in the present parasite multinucleate sporonts are formed which give rise to spores in two ways, either by directly breaking up into sporoblasts, or by the formation of pansporoblasts.

In some Microsporidia sporonts are formed from uninucleate schizonts following fission of multinucleate schizonts and later the sporont nucleus divides. Gibbs (1953) has described a species of *Gurleya*, where sporogony commences with the formation of uninucleate sporonts following binary fission of vegetative forms. In the present parasite, however, since no uninucleate forms distinct from the early schizonts have been observed, it is considered that multinucleate schizonts transform directly into multinucleate sporonts in which the nuclei may then further divide. Such a transformation has been described in other Microsporidia, for example, in a *Plistophora* found in *Periplaneta orientalis* by Mercier (1908).

It is difficult, on account of the light staining of the cytoplasm and its vacuolation, to determine how many spores are formed from a single sporont where the latter breaks up directly to form sporoblasts. When pansporoblasts are formed, however, the number of spores formed from a single sporont can be ascertained. Although some pansporoblasts with less than sixteen constituent sporoblasts are seen, the majority are composed of sixteen or more sporoblasts. It is concluded that a single sporont gives rise to an inconsistent, large (generally more than sixteen) number of spores, and the microsporidian described here is accordingly placed in the genus *Plistophora* (Jírovec, 1936). It does not appear to have been described hitherto and as it has been found only in Scatopsids the name *P. scatopsi* sp.nov. is proposed for it. The main characters of the new species (see Fig. 53) are: Uninucleate schizonts appear in the intestinal wall of the host larvae 9–14 days after ingestion of the infective spores. Schizogony by binary or multiple fission. Schizonts with up to sixteen nuclei formed. Schizonts give rise directly to multinucleate sporonts. Mature sporonts may have over fifty nuclei. Two types of spore

formation: (i) direct breaking up of multinucleate sporont, without constriction of sporont cytoplasm, to form uninucleate sporoblasts; (ii) formation of pansporoblasts by migration of nuclei to periphery and constriction of cytoplasm. Mature spores pear-shaped, 4–6 by 2–3$\mu$. Transparent membrane. Uninucleate sporoplasm forms crescent-shaped central girdle in spore. Posterior vacuole. Anterior polar cap. Polar filament, arising from polar cap, coiled within whole area of spore, surrounded by sporoplasm. No polar capsule. Mature spores occur in all stages of the host *Scatopse notata* and are probably liberated only on the death of the host fly.

## SUMMARY

1. The morphology and life cycle of a new microsporidian, parasitic in the midgut epithelium and caeca of the larvae of *Scatopse notata*, and also present in the pupae and flies, is described.

2. The name *Plistophora scatopsi* sp.nov. is proposed.

The author's thanks are due to Dr P. Tate for his constant encouragement and helpful suggestions throughout this work, which was carried out while the author was in receipt of an Animal Health Trust Research Training Scholarship.

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# THE NERVOUS SYSTEM OF *THELASTOMA BULHÕESI* (MAGALHÃES, 1900; TRAVASSOS, 1929), A NEMATODE PARASITIC IN COCKROACHES

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(With 3 Figures in the Text)

The nervous system of the female of *Thelastoma bulhõesi* (Magalhães, 1900; Travassos, 1929) has been studied by means of whole-mount preparations of living and heat-killed nematodes, stained whole mounts and by means of longitudinal and transverse sections. Stained whole mounts were prepared by fixing and staining the nematodes in Chang's nerve stain (Gray, 1954) for 24 hr. This stain consists of thionin (0·3 g.), 40 % formaldehyde (10 ml.) and distilled water (90 ml.). The nematodes were transferred to hot lactophenol until cleared and excess stain removed, then taken up to glycerine using the rapid method of Baker (1953). In these nematodes this stain, which fades after several weeks, acts more as a background stain to show up the nerves than as a nerve stain. Feulgen-stained whole mounts were prepared following Bergan's (1955) technique for trematodes. The nematodes were killed and fixed in Gilson's fluid for 15 min., washed in distilled water for 10 min., rinsed in N/1 HCl for 5 min., hydrolysed in N/1 HCl at 60° C. for 10 min., rinsed in cold N/1 HCl, washed in distilled water for 10 min., stained in De Tomasi's modification of Schiff's reagent (Pearse, 1953) for 4 hr., rinsed in three changes of bisulphite solution (10 min. in each), washed in distilled water for 5 min., dehydrated in alcohol, cleared in xylene and mounted in DPX. The nuclei, including those of the nervous system, stain reddish purple. Chromosomes present in the embryonating eggs inside the females were also clearly stained by this method. Sections of paraffin-embedded material were stained with haematoxylin and eosin or with 1 % toluidine blue.

The central nervous system (Fig. 1) is similar to those of nematodes studied by other workers. The nerve ring encircles the oesophagus and is situated mid-way between the mouth and the base of the oesophagus, with the dorsal part of the ring slightly anterior to the ventral part. Each of the six cephalic papillary nerves possesses a ganglion close to its junction with the nerve ring, but these ganglia are not compact groups of cells (Fig. 3). The anterior parts of the cephalic papillary nerves are obscured by three granular lobed structures which extend from the stoma half-way towards the nerve ring (Fig. 1). These granular structures, which stain heavily with haematoxylin and are quite distinct in the living animal, appear to be arcade cells which have either fused or are the remnants of the usual number of nine cells found in those nematodes which possess arcade cells (Martini, 1916; Mackin, 1936; Chitwood & Chitwood, 1933, 1950). Two of these structures are

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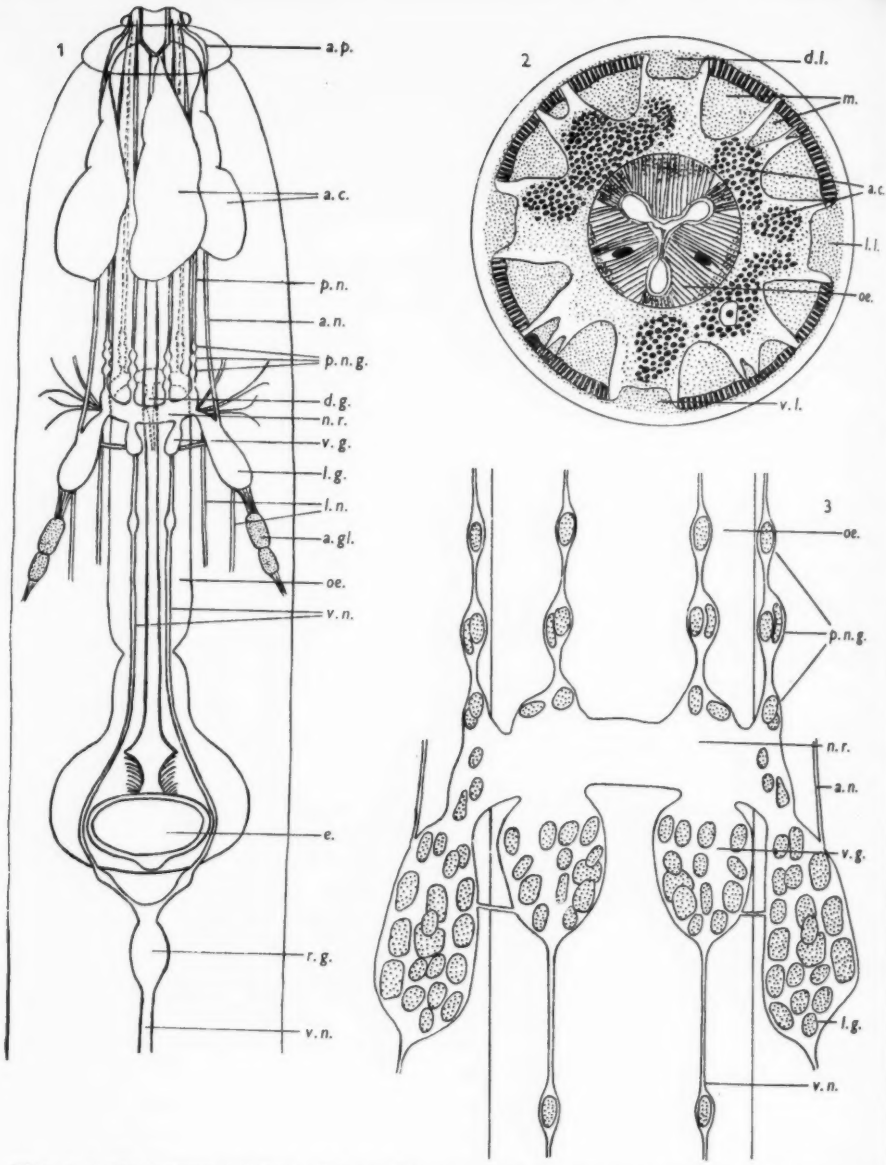


Figs. 1–3. *Thelastoma bulhõesi*. Fig. 1. Diagrammatic representation of the female nervous system (ventral view). Fig. 2. Transverse section of the female in the region of the arcade cells. Fig. 3. Diagram showing the distribution of nuclei in the ventral, lateral and cephalic papillary ganglia (ventral view).

| | |
|---|---|
| *a.c.* | arcade cell |
| *a.gl.* | amphidial gland |
| *a.n.* | amphidial nerve |
| *a.p.* | amphidial pouch |
| *d.g.* | dorsal ganglion |
| *d.l.* | dorsal line |
| *e.* | excretory pore |
| *l.g.* | lateral ganglion |
| *l.n.* | lateral nerve |
| *l.l.* | lateral line |
| *m.* | muscle cell |
| *n.r.* | nerve ring |
| *oe.* | oesophagus |
| *p.n.* | papillary nerve |
| *p.n.g.* | papillary nerve ganglion |
| *r.g.* | retrovesicular ganglion |
| *v.g.* | ventral ganglion |
| *v.l.* | ventral line |
| *v.n.* | ventral nerve |

situated dorso-laterally and the third is situated ventro-laterally (Fig. 2). These arcade cells, which are also found in the mature male but not in the female fourth-stage larva, have not been mentioned in earlier descriptions of *Thelastoma bulhõesi* and it is possible that this is a subspecies of *T. bulhõesi*. The amphidial nerves have been traced from the lateral ganglia to the amphidial pouch near the base of the stoma. From this pouch the amphidial duct leads to the amphidial pore.

The dorsal ganglion is small and appears to possess only one ganglion cell. Chitwood & Chitwood (1933) found only one ganglion cell in the dorsal ganglion of *Cephalobellus papilliger*, whilst Martini (1916) found none in *Oxyuris equi*; however, most other species of nematode examined contained two nerve cells. The dorsal nerve is a thin structure embedded in the hypodermis. There are no sub-dorsal ganglia.

The lateral ganglia of *Thelastoma* each contain nineteen cells (Fig. 3). Goldschmidt (1908) found thirty-five nerve cells in the lateral ganglion of *Ascaris*; Martini (1916) found twenty-one in *Oxyuris*; Chitwood & Chitwood (1933, 1950) found twenty-six in *Cephalobellus*, forty-two in *Spironoura* and forty-one in *Oesophagostomum*; whilst Chitwood & Wehr (1934) found forty-two in *Rhabditis*. Posterior to the lateral ganglia are two granular structures which may be amphidial glands (Fig. 1). A minute commissure joins the lateral ganglion to the ventral ganglion.

In *Thelastoma*, as in *Oxyuris*, the ventral ganglion is definitely paired but each ganglion contains thirteen cells (Fig. 3), whereas those of *Oxyuris* each contain eight cells (Martini, 1916). The ventral nerve arises from the two ventral ganglia as a paired structure and in each nerve, slightly posterior to the ventral ganglion, there arises a swelling containing a nucleus (Figs. 1, 3). These nerves are paired to the posterior side of the excretory pore where they fuse in the retrovesicular ganglion, composed of thirteen cells as in *Ascaris*, *Oxyuris* and *Cephalobellus*, and appear to continue as a single nerve. On each of the ventral nerves, slightly anterior to the retrovesicular ganglion, there is a swelling containing a nucleus (Fig. 1). The ventral nerve has groups of cells situated along its length, with large groups forming a post-vulval ganglion and a pre-anal ganglion. Martini (1916) found the ventral nerve of *Oxyuris equi* to be paired to the posterior side of the vulva but the ventral nerve of *Thelastoma bulhõesi* passes to the right of the vulva as in all of the species studied by other workers. In *T. bulhõesi*, mid-way between the post-vulval ganglion and the pre-anal ganglion, the ventral nerve becomes a paired structure with the left nerve much thinner than the right; however, they fuse again just before entering the pre-anal ganglion.

There appear to be several lateral nerves which communicate with the ventral nerve by means of commissures but these lateral nerves have not been traced.

Although the nervous system of *T. bulhõesi* follows the general plan found in all other nematodes studied there are several minor differences. These include the single nerve cell in the dorsal ganglion instead of two as in most other species studied; the number of nerve cells found in the lateral and in the ventral ganglia and the apparent doubling of the ventral nerve between the post-vulval and pre-anal ganglia. Chitwood & Chitwood (1950) believe that because of the minute-

ness of the left ventral nerve, doubling of the ventral nerve may have been overlooked and so it is possible that the ventral nerve of *T. bulhõesi* is not only double from the nerve ring to the retrovesicular ganglion and between the post-vulval and pre-anal ganglia but throughout the entire length, fusing only at the ganglia situated along it.

## SUMMARY

The nervous system of the female of *Thelastoma bulhõesi* is described. Three granular lobed structures, presumably arcade cells, are present at the anterior end of the female and of the male.

I wish to express my thanks to Dr P. Tate for his advice and encouragement throughout this investigation. Thanks are also due to the Department of Scientific and Industrial Research for financial assistance during part of this work.

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# *NEOSPROSTONIA*, A NEW NAME FOR *SPROSTONIA* JAIN, 1959

By S. L. JAIN

*Indian Statistical Institute, Calcutta* 35

In a recent issue of *Parasitology*, I have described (Jain, 1959) a new genus of freshwater Tetraonchinae, *Sprostonia*, from the gill filaments of some Indian fishes. In a personal communication Dr Nora G. Sproston has kindly pointed out to me that the same name has been used by Bychowsky in his monograph on Monogenea (1957), published by the Institute of the Zoological Academy of Sciences of the U.S.S.R.,for *Sprostonia squatinae* (MacCallum, 1921) which he makes genotype and removes it from Trochopodinae to a new subfamily Megalocotylinae in Capsalidae. I therefore change the name of the genus which I have described to *Neosprostonia*, *Sprostonia* being preoccupied.

Further, from a personal communication from Dr A. V. Gussev (Zoological Institute, Academy of Sciences of the U.S.S.R., Moscow), I learn that the genus *Neosprostonia* has close resemblance to another genus, *Bychowsyella* Achmerow, 1952. Gussev (1955) re-examined this genus and upon comparison from his figures (p. 317), I find that in fact the two parasites are identical in generic characters. Another genus, *Silondotrema*, erected by Tripathi (1957), has come to my notice recently. It bears close identity to *Neosprostonia* and *Bychowsyella*. Thus three names appear to have been given to parasites having almost identical generic characters. The priority goes to *Bychowsyella* and the other two genera, in my opinion, may be regarded as its synonyms.

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# THE LARVAL TREMATODES OF CERTAIN FRESH-WATER MOLLUSCS

## I. THE FURCOCERCARIAE

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(With 6 Figures in the Text)

Twenty-two species of cercariae were encountered in certain fresh-water molluscs from Glamorganshire during a 3-year study. They comprise eight species of furcocercariae, which are dealt with in this paper, as well as three species of gymnocephalous and three of echinostome cercariae, five xiphidiocercariae and one species of each of the monostome, microcercous and cerceriaeum types which will be described in subsequent publications. Observations were made on the incidence, morphology, behaviour and as far as possible on the life cycles of all these species.

### METHODS OF STUDY

The molluscs were isolated in batches of six, or singly in the case of the larger molluscs, *Planorbis corneus* L. and *Lymnea stagnalis* L. An accurate determination of the degree of parasitization by mature and developmental stages was made by the dissection of the molluscs, since emergence of cercariae indicated the presence of mature forms only. Observations were made on cercariae obtained on emergence and by dissection of the mollusc. The spination and excretory system were best observed in living specimens under cover-glass pressure. A very dilute solution of neutral red was of use in bringing the gut and penetration gland cells into prominence. The addition of a small quantity of aceto-carmine to water containing several cercariae fixed and stained the specimens and rendered the genital rudiment a dark red colour and readily visible. All measurements were made with the aid of an ocular micrometer, in each case ten living and ten fixed emerged cercariae were measured and hot 10% formalin was the fixative used.

### DESCRIPTIONS

The classification followed in this paper is that outlined by Miller (1926). Of the eight species of furcocercariae described here, four are new, two have previously been reported from Europe but not from Britain, and the remaining two species have been described from Glamorganshire by Rees (1932).

### A. Pharyngeal longifurcous distome cercariae

#### (1) **Cercaria tetraglandis** n.sp. (Fig. 1)

*Morphology*

Relatively small cercaria. Body length greater than both tail-stem length and furcal length. Body tapering anteriorly and rounded posteriorly, with greatest

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width in region of ventral sucker. Anterior penetration organ oval, well developed and muscular. Rounded ventral sucker projecting slightly from body surface. Two pairs of small anterior penetration-gland cells closely applied to margin of ventral sucker. Penetration-gland ducts running antero-laterally with convolutions

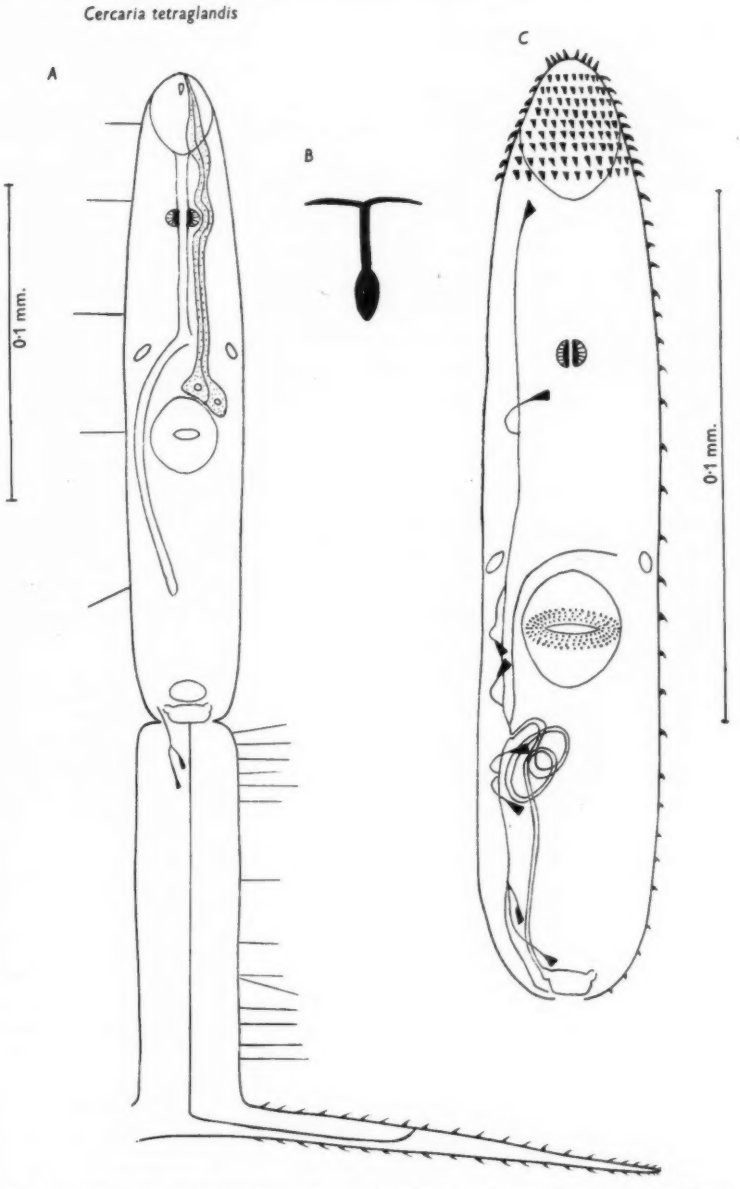


Fig. 1. *Cercaria tetraglandis* n.sp. *A*, General structure; *B*, resting position; *C*, spination and excretory system.

in region of pharynx and dilations within anterior organ opening anterior and lateral to mouth. Subterminal mouth, relatively long prepharynx, well-developed muscular pharynx situated almost midway between anterior organ and ventral sucker. Short thin-walled oesophagus bifurcating some distance anterior to the ventral sucker into two long thin-walled caeca terminating just anterior to excretory bladder. Caeca wall refractile and sometimes appearing septate. Small trilobed excretory bladder. Main tubules arising antero-laterally and forming a refractile knot posterior to ventral sucker. Primary duct here dividing into anterior and posterior secondary tubules. Transverse commissure anterior to acetabulum. Anterior tubule draining four flame cells and posterior receiving capillaries of six, four of which lie within the body and two in the tail. Caudal flame cells asymmetrically placed in proximal portion of tail stem. Excretory tubule traversing tail stem in mid-line and bifurcating posteriorly, each branch passing into a furca and opening midway along margin. Unpigmented eye spots anterior to ventral sucker. Genital rudiment not easily visible in living specimens but in form of small rounded mass of discrete cells anterior to bladder in specimens treated with aceto carmine. Tail stem with crenulate margin, well-developed musculature, numerous refractile nuclei and small irregular caudal bodies scattered throughout.

*Spination*

Eight forwardly directed penetration spines. Bare circum-oral patch. Nine conspicuous rows of spines on proximal half of anterior organ, less conspicuous spines, also arranged transversely, extending to region posterior to ventral sucker. Fewer, irregularly scattered spines on posterior part of the body. Four rows of spines on ventral sucker. Tail-stem aspinose, furcae spinose. Fourteen pairs of fine hair-like structures on tail stem and five pairs on body.

*Measurements* (mm.)

| | Living | | Fixed | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| B.L. | 0·11–0·23 | 0·162 | 0·150–0·175 | 0·165 |
| B.B. | 0·05–0·08 | 0·065 | 0·030–0·035 | 0·031 |
| T.S.L. | 0·09–0·13 | 0·10 | 0·110–0·115 | 0·113 |
| T.S.B. | 0·03 | 0·030 | 0·025–0·030 | 0·029 |
| F.L. | 0·10–0·17 | 0·140 | 0·130–0·140 | 0·135 |
| F.B. | 0·02–0·025 | 0·022 | 0·010 | 0·010 |
| O.S. | 0·02–0·035 × 0·015–0·02 | 0·026 × 0·019 | 0·020–0·030 × 0·010–0·015 | 0·024 × 0·014 |
| V.S. | 0·02–0·03 × 0·02–0·03 | 0·028 × 0·029 | 0·015–0·020 × 0·015–0·030 | 0·019 × 0·016 |

*Sporocyst*

*Daughter sporocyst.* In digestive gland of mollusc host. Long, filiform sporocyst with anterior, subterminal birth pore. Actively contractile. Tapering anteriorly and rounded posteriorly. Thin-walled with solid mass of cells at anterior and posterior poles. Numerous constrictions. Difficult to separate completely from digestive gland.

*Measurements* (mm.)

| | Living (ten specimens) | | Fixed (five specimens) | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| L. | 2·75–8·75 | 5·70 | 3·48–8·77 | 6·00 |
| B. | 0·14–0·36 | 0·23 | 0·19–0·24 | 0·21 |

*Mother sporocyst*

At base of visceral mass. Terminal birth pore. Opaque and often pigmented. Containing numerous daughter sporocysts. A portion of a sporocyst fixed measured L. 6·64 mm. B. 2·7 mm. Distinguished from daughter sporocyst by greater width, position of birth pore, nature of contents and opacity.

*Emergence and behaviour*

The cercariae emerge in large numbers and tend to aggregate on the side of the tube furthest from the light source. They are emitted from the snail host throughout the day but most heavily in the early afternoon. Short bursts of swimming are followed by periods of rest in which the body and tail stem hang downwards and the furcae are held horizontally but may be slightly curved. After decaudation the body may live for some hours and can be found creeping on the bottom of the tube. The cercariae live for approximately 24 hr. at room temperature.

*Host*

*Planorbis corneus* L.

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Small, pharyngeal, longifurcous, distome furcocercaria. Two pairs of anterior penetration-gland cells. Flame-cell formula $2(4+4+(2)) = 20$. Anterior excretory commissure. Unpigmented eye spots. Eight penetration spines. Tail-stem aspinose, furcae spinose. Fourteen pairs of hair-like structures on tail stem and five pairs on body. Ventral sucker bearing four rows of spines.

*Further development*

The cercariae penetrate into aquatic oligochaetes and leeches. It is doubtful whether development would be completed within the oligochaetes, but it is known that several species of fresh-water leeches feed on oligochaetes and this may be a secondary route of infection. The development into a tetracotyle metacercaria inside certain species of hirudineans has been followed and will be dealt with in a subsequent paper. Attempts to complete the life cycle, using laboratory-bred ducklings as the final host, have been unsuccessful.

*Comparison with related species*

Eleven species of cercariae share with *C. tetraglandis* an identical penetration-gland cell number and arrangement and flame-cell formula. They are: *C. hysteromorpha triloba* (Hugghins, 1954); *C. flabelliformis* (Oliver & Cort, 1941); *C. douglasi* (Cort & Brooks, 1928); *C. sanjuanensis* (Miller, 1926); *C. helvetica* XXXIV (Dubois, 1929); *C. laticaeca* and *C. berghei* (Fain, 1953); *C. mudaliari* (Peter, 1953); *C. stonii* (Brooks, 1943); *C. tetradena* (Johnston & Beckwith, 1945); *C. cotylurus cornutus* syn. *C. A. Szidat*, *C. strigea tarda*, (?) *C. sanjuanensis* (Dubois, 1929, Komiya, 1938; Mathias, 1925; Harper, 1931). *C. samburgi* (Goodman, 1951) and *C. sudanensis* no. 5 (Archibald & Marshall, 1932) have the same penetration-gland cell number, but the flame-cell formulae are not known. All these may be differentiated from *C. tetraglandis* as follows:

*C. samburgi* and *C. sudanensis* no. 5 although incompletely described may be differentiated from *C. tetraglandis* on the structure of the gut, the relatively greater tail-stem length and the position of the opening of the furcal excretory tubules, terminal in *C. samburgi*, and subterminal in *C. sudanensis* no. 5. *C. samburgi* may also be distinguished from *C. tetraglandis* on the basis of spination, although it has a similar resting position.

*C. helvetica* XXXIV, *C. c. cornutus*, *C. laticaeca*, *C. berghei*, *C. mudaliari* and *C. tetradena* may be differentiated on the presence of large caudal bodies within the tail stem of newly emerged cercariae. In this connexion it is important that the cercariae are examined soon after emergence as Erasmus (1954) has shown that the caudal bodies represent a glycogen store which becomes used up during the life of the cercaria, so that they become diffuse and scattered some time after emergence.

*C. tetradena* and *C. c. cornutus* also differ from *C. tetraglandis* in that the furcal excretory tubules open at the tip. *C. hysteromorpha triloba* and *C. laticaeca* have a different resting position. *C. stonii*, *C. h. triloba*, *C. douglasi*, *C. sanjuanensis*, *C. c. cornutus*, *C. flabelliformis* and *C. helvetica* XXXIV differ in details of spination. It is of interest to note here that *C. flabelliformis* differs from *C. douglasi* chiefly on the basis of measurements, (Oliver & Cort, 1941). *C. h. triloba*, *C. douglasi*, *C. flabelliformis* and *C. helvetica* differ from *C. tetraglandis* in the relative proportions of the tail stem and furcae, and *C. stonii*, *C. c. cornutus* and *C. sanjuanensis* in their larger size. *C. c. cornutus* differs further in the absence of unpigmented eye spots, and *C. sanjuanensis* also differs in that the anterior commissure possesses blind branches. *C. c. cornutus*, *C. laticaeca* and *C. douglasi* differ from *C. tetraglandis* also in the structure of the gut and *C. h. triloba* differs further in the position of the caudal flame cells.

### (2) **Cercaria paracauda** sp.nov. (Fig. 2)

Body length approximately equal to that of tail stem and of furca. Body tapering anteriorly and truncate posteriorly. Greatest width in region of acetabulum. Well developed, oval, muscular anterior penetration organ. Ventral sucker slightly posterior to middle of body, well developed and conspicuous. Four large penetration-

gland cells posterior to ventral sucker, easily discernible and with clear refractile nuclei. Large sinuous penetration-gland cell ducts running antero-laterally with dilations anteriorly and within anterior organ, opening on margin of anterior organ. Mouth subterminal, short thin-walled prepharynx, conspicuous, muscular

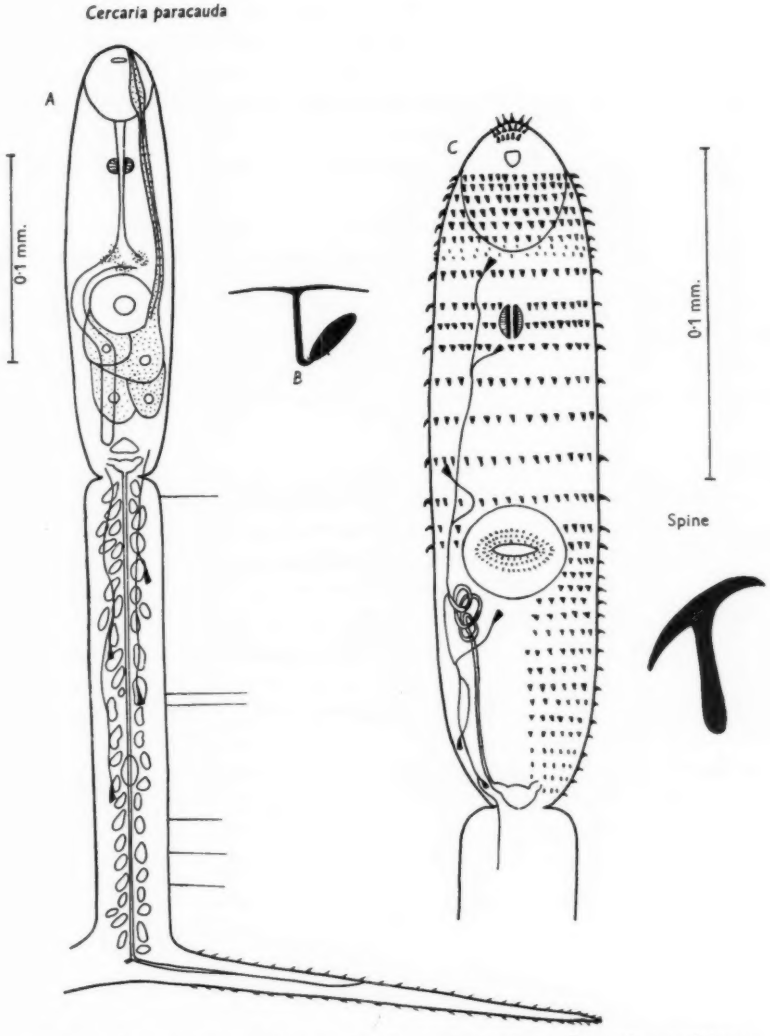


Fig. 2. *Cercaria paracauda* n.sp. *A*, General structure; *B*, resting position; *C*, spination and excretory system.

pharynx, long, thin-walled, delicate oesophagus bifurcating at anterior margin of ventral sucker into two wide caeca which extend to excretory bladder. Large caeca most conspicuous part of living and fixed material. Small, tripartite

excretory bladder, primary collecting canals arising antero-laterally and posterior to ventral sucker show complex convolutions forming refractile coil containing two ciliated patches. Primary canal then dividing into anterior and posterior collecting tubules. Each anterior tubule draining three flame cells and each posterior tubule receiving capillaries of three flame cells situated in the posterior portion of the body and two within the anterior two-thirds of the tail stem. Caudal flame cells asymmetrically arranged. No excretory commissure or island of Cort observed. Caudal excretory tubule arising from median chamber of bladder traversing tail stem in mid-line and dividing distally into two tubules, each passing into a furca and opening midway along margin. Genital rudiment small triangular mass of nuclei anterior to excretory bladder, visible only in fixed and stained specimens. Eye spots absent.

Tail stem with well-developed musculature enclosing numerous refractile nuclei and small caudal bodies. The latter irregularly arranged and adhering to caudal excretory tubule, those in proximal portion larger than those in distal region. Some of the latter may pass into furcae during movement. Furcae characteristic in possessing well-defined attenuated tip.

A diagnostic point of this larva is the presence of yellow pigment granules around the point of origin of the intestinal caeca.

*Spination*

Three rows of forwardly directed penetration spines anterior to oral opening, first row comprising five spines, second seven, and third five spines. Bare circum-oral patch followed by oral cap of six, conspicuous, closely set, transverse row of relatively large, backwardly projecting spines on middle third of anterior organ. Less conspicuous rows of spines more widely set on distal portion of penetration organ. Ventrally, ten conspicuous and widely spaced transverse rows of large spines extending from posterior part of penetration organ to anterior margin of ventral sucker. Less conspicuous spines posterior to ventral sucker. Furca spinose, tail-stem aspinose bearing six pairs of slender hair-like projections. One pair of hair-like structures on posterior part of body. Ventral sucker bearing three rows of spines, differing in size, with longest spines comprising outer row and shortest the inner row.

*Measurements* (mm.)

| | Living | | Fixed | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| B.L. | 0·14–0·27 | 0·204 | 0·19–0·24 | 0·208 |
| B.B. | 0·06–0·09 | 0·070 | 0·04–0·06 | 0·049 |
| T.S.L. | 0·16–0·25 | 0·210 | 0·19–0·24 | 0·220 |
| T.S.B. | 0·04–0·06 | 0·049 | 0·03–0·04 | 0·037 |
| F.L. | 0·17–0·26 | 0·230 | 0·19–0·24 | 0·220 |
| F.B. | 0·02–0·03 | 0·028 | 0·01–0·02 | 0·019 |
| ANT.ORG. | 0·06–0·09 × 0·03–0·05 | 0·069 × 0·047 | 0·03–0·04 × 0·03 | 0·039 0·030 |
| V.S. | 0·03–0·04 × 0·04 | 0·039 × 0·040 | 0·03 × 0·03–0·04 | 0·030 × 0·033 |

*Sporocyst*

Long and filiform with few constrictions. Subterminal birth pore surrounded by characteristic muscular thickening, difficult to separate from digestive gland. Anterior end tapering and rounded posteriorly. Three entire living sporocysts containing cercariae and germ balls varied in length between 0·24 and 0·325 mm. and in breadth between 0·12 and 0·19 mm.

*Emergence and behaviour*

The cercariae emerge throughout the day, but more particularly in the early morning and midday. They tend to aggregate in the upper levels of the water. In the resting position, the furcae are spread horizontally and the tail stem is bent in the proximal third so that the body is held at an acute angle.

*Host.*

*Lymnea pereger* Mull.

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Pharyngeal, longifurcous, distome cercaria. Two pairs of penetration cells posterior to ventral sucker. Flame-cell formula $2(3+3+(2)) = 16$. No excretory commissure. Two ciliated patches in refractile coil of primary tubule, posterior to ventral sucker. No eye spots. Three rows of penetration spines. Ten conspicuous, widely spaced, transverse rows of spines between anterior organ and ventral sucker. Furcae spinose, tail stem aspinose with six pairs of tail-stem hairs. Single pair of hair-like structures on posterior part of body.

*Further development*

The cercariae have been observed to penetrate into three- and ten-spined sticklebacks. In one case where a stickleback was exposed to infection for ½ hr. and dissected 2½ hr. later it was found that there was one cercarial body in the humours of each eye and eight within the lens. These cercariae develop into diplostomuli within the lens.

*Comparison with related species of cercariae*

Only nine cercariae have previously been described with a flame-cell formula and penetration-gland cell number and arrangement identical with those of *C. paracauda*. They are: *C. yogena* (Cort & Brackett, 1937); *C. laruei, C. flexicauda* (Cort & Brooks, 1928); *C. diplostomum spathaceum* (syn. *C. C. Szidat*) (Komiya, 1938); *C. d. phoxini* (Rees 1957); *C. maritzburgensis* (Porter 1938); *C. helvetica* XIII and *C. helvetica* XV (Dubois, 1929) and *C. murrayensis* (Johnston & Cleland, 1938).

*C. tuberistoma* and *C. gracillima* (Faust, 1917) possess two pairs of penetration-

gland cells posterior to the ventral sucker but no details of the excretory system are given and the descriptions are insufficient to allow comparative discussion.

*C. modicella* (Cort & Brooks, 1928) possesses four posterior penetration-gland cells, but a flame cell formula different from that of *C. paracauda* and is further distinguished on its different resting position, the presence of only six pairs of caudal bodies and ten penetration spines. *C.* sp. (Hesse, 1923) has a penetration-gland cell number similar to *C. paracauda*, but the flame-cell formula is $2(7+(2)) = 18$ and therefore it may readily be distinguished from the latter species. The nine species listed above may be distinguished from *C. paracauda* on the basis of the following data.

*C. diplostomum spathaceum* may be distinguished from *C. paracauda* on the presence of only two rows of spines on the ventral sucker, blind ending ducts anterior to the ventral sucker, and an island of Cort.

*C. yogena* is a smaller species and further differs from *C. paracauda* in its resting position, in which the body and tail stem hang downward with the preacetabular part of the body bent slightly ventral, in the presence of only twelve large caudal bodies and an island of Cort, in its possessing only twelve penetration spines and in the absence of distinct rows of spines on the body. Yellow pigment is also present in *C. yogena*, but is not restricted to the bases of the intestinal caeca but may extend to the oral sucker, posterior to the ventral sucker and even into the tail stem. This species has been observed to penetrate into sunfish but its development has not been followed.

*C. laruei* and *C. flexicauda* have the same resting position as *C. paracauda*, and similarly penetrate into fish and localize within the lens of the eye. They differ from *C. paracauda* in that in both the tail stem and furcae are proportionately much larger than the body. Further, *C. laruei* possesses only fifteen penetration spines which are not arranged in rows. *C. flexicauda* and *C. helvetica* XIII, which according to Dubois (1929) differ from each other only in the measurements of the various parts, may be distinguished from *C. paracauda* on the presence of only two rows of spines on the ventral sucker, ten penetration spines and the smaller number of caudal bodies. The daughter sporocysts of *C. helvetica* XV, *C. yogena* and *C. murrayensis* differ from those of *C. paracauda* in that the birth pore is antero-lateral. *C. murrayensis* differs further in that it is a smaller species and in details of spination; there are twelve penetration spines in a single row and six double rows of spines on the body between the anterior organ and the hind margin of the ventral sucker and only two rows of spines on the ventral sucker. Furthermore, the presence of an island of Cort and the absence of ciliation in the primary excretory tubules distinguishes *C. murrayensis* from *C. paracauda*, but they are similar in the restriction of the yellow pigment to the point of origin of the intestinal caeca.

*C. maritzburgensis* is distinguished from *C. paracauda* on the presence of only six pairs of caudal bodies, the furcal tubules opening at a point about three-quarters the way along the margin of the furcae, and the caudal flame cells being symmetrically placed. It may also be differentiated from *C. paracauda* on details of spination; the oral cap comprises ten rows of spines and there are fifteen widely

spaced, conspicuous, transverse rows on the body. *C. helvetica* XV is characterized by the presence of furcal fin folds and fewer caudal bodies, and differs in details of spination; there are only two rows of penetration spines, the oral cap comprises four or five rows of spines, there are only two rows of spines on the ventral sucker and double rings of spines on the body, the tail stem is spinose but bears six pairs of tail stem hairs similar to *C. paracauda*. *C. d. phoxini* has only six pairs of caudal bodies, twelve pairs of tail stem hairs, only five penetration spines and two rows of spines on the ventral sucker all of which distinguish it from *C. paracauda*.

On the basis of the above comparison with related species it is apparent that *C. paracauda* is a new species.

The nine previously described species listed above with an identical penetration-gland cell system and flame-cell formula, together with *C. paracauda* form a closely related group of cercariae, very similar in their morphology and biology. They are all further characterized by the large and conspicuous intestinal caeca and in all cases, where the subsequent development is known (i.e. all except *C. helvetica* XIII and *C. helvetica* XV) the cercariae penetrate into fish and localize within the lens. The specific differences between these nine species of cercariae are based on spination, size, number of caudal bodies and the resting position. The validity and significance of these characters can only be assessed when further details of the life cycles of these cercariae are known.

These cercariae are obviously related to another group comprising *C. X.* (Rees, 1932) *C. chromatophora* (Brown, 1931) and *C. chrysenterica* (Miller, 1926) which possess an identical penetration-gland cell number and arrangement and also develop into diplostomuli within the lens of fishes but have the flame-cell formula $2(3+4+(2)) = 18$.

Erasmus (1954) has suggested that *C. X.* and *C. chromatophora* are synonymous.

### (3) **Cercaria planorbida** n.sp. (Fig. 3)

*Morphology*

Body shorter than both tail stem and furcae. Tail stem thick, furcae slightly constricted off from base of tail stem. Body tapering anteriorly and rounded posteriorly. Well-developed anterior penetration organ. Ventral sucker muscular and situated posterior to middle of body. Three pairs of posterior penetration gland cells. Most anterior pair closely applied to posterior margin of ventral sucker. Penetration-gland cell ducts closely applied to lateral margins of ventral sucker and gut and exhibiting dilations within anterior organ. Subterminal mouth, thin-walled short prepharynx, muscular, well-developed pharynx, long delicate oesophagus bifurcating immediately in front of ventral sucker, short intestinal caeca not extending beyond most posterior of penetration-gland cells, with distal portions curving inwards. Caeca often appearing septate. Small spherical bladder with small antero-lateral dilations into which primary excretory tubules open. These canals extending laterally and posteriorly to ventral sucker forming a refractile coil before dividing into anterior and posterior secondary collecting tubules. A posterior excretory commissure. Each anterior tubule draining two flame cells and each posterior receiving capillaries of four flame cells situated in

the posterior part of the body, and one in the proximal region of tail stem. Caudal flame cells asymmetrically placed. Median caudal tubule dividing distally into furcal canals which open midway along margin of furcae. Unpigmented eye spots anterior and lateral to ventral sucker. Genital rudiment represented by rounded

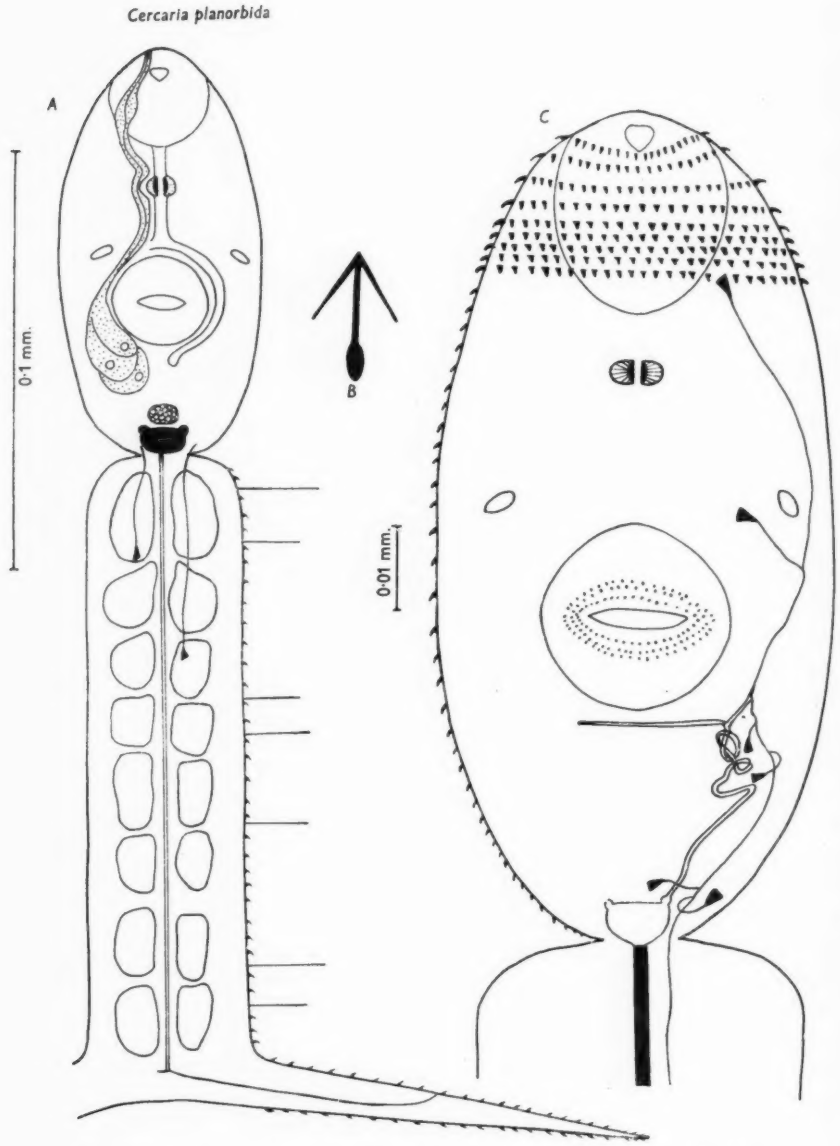


Fig. 3. *Cercaria planorbida* n.sp. *A*, General structure; *B*, resting position; *C*, spination and excretory system.

mass of nuclei anterior to the excretory bladder, readily distinguished only in fixed and stained specimens. Tail stem containing eight pairs of caudal bodies often irregularly arranged. Well-developed longitudinal and circular muscles in tail stem.

*Spination*

Single row of penetration spines anterior to mouth, circumoral bare patch. Cap of nine closely set rows of spines on proximal part of anterior organ followed by more widely spaced rows of spines to region of ventral sucker. Furcae spinose. Tail stem spinose bearing seven pairs of delicate, refractile, hair-like structures. Ventral sucker bears three rows of spines.

*Measurements* (mm.)

| | Living | | Fixed | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| B.L. | 0·1–0·21 | 0·157 | 0·0935–0·099 | 0·0985 |
| B.B. | 0·06–0·090 | 0·067 | 0·044–0·0495 | 0·047 |
| T.S.L. | 0·12–0·2 | 0·16 | 0·12–0·148 | 0·140 |
| T.S.B. | 0·03–0·06 | 0·049 | 0·038 | 0·038 |
| F.L. | 0·14–0·19 | 0·16 | 0·115–0·13 | 0·126 |
| F.B. | 0·025–0·03 | 0·029 | 0·01–0·016 | 0·015 |
| ANT.ORG. | 0·03–0·08 × | 0·041 × | 0·022–0·033 × | 0·024 × |
| | 0·03–0·04 | 0·032 | 0·02–0·03 | 0·024 |
| V.S. | 0·04 × | 0·04 × | 0·02 × | 0·02 × |
| | 0·04 | 0·04 | 0·02–0·027 | 0·023 |

*Sporocyst*

In digestive gland of mollusc host. Subterminal birth pore.

*Measurements* (mm.) (five specimens)

| | Living | | Fixed | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| L. | 0·36–1·74 | 1·126 | 1·35–2·51 | 1·72 |
| B. | 0·09–0·15 | 0·128 | 0·09–0·13 | 0·121 |

*Behaviour and emergence of cercariae*

The cercariae emerge in the early morning and throughout the day. They tend to aggregate at the base of the tube or the side of the vessel furthest from the source of light. During the resting position the furcae are held at an acute angle to the tail stem which together with the body hangs directly downward.

*Host*

*Planorbis planorbis.*

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Longifurcate, pharyngeal, distome cercaria. Six posterior penetration-gland cells. Excretory formula $2(2+4+(1)) = 14$. Unpigmented eye spots. Posterior excretory commissure. Eight pairs caudal bodies. Single row of penetration spines. Furcae spinose. Tail stem spinose with seven pairs of hair-like structures. Resting position in which body and tail stem hang directly downward but the furcae are held at an acute angle to the stem.

*Further development*

Unknown. The cercariae were observed to attach to leeches but not to penetrate.

*Comparison with related species*

The only species previously described with a penetration-gland cell number and arrangement and flame-cell formula identical with that of *C. planorbida* is *C. riponi* (Brackett, 1939). This may be distinguished from *C. planorbida* on its different resting position in which the furcae are held horizontally, the presence of only six or seven pairs of caudal gland cells and the anterior bifurcation of the oesophagus. It differs further in the presence of a posterior group of ducts in non-emerged cercariae and in details of spination; the furcae are aspinose, penetration spines are absent and there are three or four rows of spines on the ventral sucker. These two species are alike in the presence of unpigmented eye spots, short intestinal caeca and a posterior excretory commissure. *C. wardlei* (Miller, 1926) has six posterior penetration-gland cells and the same flame-cell number but a different arrangement $2(3+3+(1)) = 14$ and may therefore be readily distinguished.

### (4) **Cercaria apatemon gracilis minor** (Yamaguti, 1933) (Fig. 4)

Syn. *C. hamburgensis* (Komiya, 1938)

? *C. pygocytophora* (Brown, 1931)

It was found during this study that a species of cercaria very similar to that described by Komiya (1938) as *C. hamburgensis* was in fact the larva of *C. apatemon gracilis minor*, Yam. Slight differences exist between the two species in measurements, namely, the body length, furcal breadth and the size of the ventral sucker which may be accounted for by differences in the state of contraction and expansion of the living material and cover glass pressure. Unfortunately, Komiya gave the measurements of living specimens only, which are more variable than those of fixed specimens. Other differences are the presence in *C. hamburgensis* of an island of Cort and only two or three rows of spines on the ventral sucker, whereas I observed four in my specimens. Komiya did not give any other details of spination and these are given below with a more detailed description of the morphology of the cercaria. It is unlikely that *C. hamburgensis* and *C. a. g. minor* are in fact distinct species. If on re-examination of *C. pygocytophora* a posterior commissure is found, it may also prove to be synonymous with *C. a. g. minor*.

*Morphology*

Relatively small cercaria with four posterior penetration-gland cells. Furcal length and tail-stem length exceed that of body. Subterminal mouth, short prepharynx, muscular pharynx and long oesophagus bifurcating immediately anterior to ventral sucker into two caeca which terminate a short distance behind the posterior margin of the ventral sucker. Small spherical excretory bladder. Posterior to ventral sucker, primary collecting tubules forming a refractile coil before dividing into anterior and posterior collecting canals. Posterior excretory commissure and anterior blind ending ducts arising in this region. Two pairs of anterior flame cells, four pairs posterior and one pair in proximal portion of tail stem. Median caudal excretory tubule dividing distally into furcal tubules which open midway along anterior margin of furcae. Unpigmented eye spots present. Oval mass representing genital rudiment anterior to excretory bladder visible only in fixed and stained specimens. Seven pairs of caudal bodies present in tail stem, also a small caudal body at the base of each furca.

*Spination*

No penetration spines. Circumoral spineless area. Eight closely set rows of backwardly directed spines covering anterior third of penetration organ followed by five more widely set rows. Smaller spines arranged in transverse rows on remainder of body. Furcae spinose but tail stem aspinose. Four concentric rows of hooks on ventral sucker. One pair of hair-like projections on body posterior to the ventral sucker and eleven pairs on the tail stem.

*Measurements* (mm.)

| | Living | | Fixed | | | |
|---|---|---|---|---|---|---|
| | Author's | | Komiya | | Author's | |
| | Range | Average | Range | Average | Range | Average |
| B.L. | 0·11–0·2 | 0·173 | 0·113–0·128 | 0·121 | 0·11–0·12 | 0·116 |
| B.B. | 0·03–0·08 | 0·055 | 0·055–0·063 | 0·057 | 0·03–0·04 | 0·038 |
| T.S.L. | 0·12–0·13 | 0·124 | 0·150–0·177 | 0·158 | 0·12–0·14 | 0·143 |
| T.S.B. | 0·04–0·05 | 0·045 | 0·045–0·053 | 0·048 | 0·03–0·04 | 0·039 |
| F.L. | 0·11–0·15 | 0·129 | 0·113–0·130 | 0·125 | 0·12–0·138 | 0·135 |
| F.B. | 0·02–0·03 | 0·028 | 0·015–0·019 | 0·017 | 0·016–0·02 | 0·0167 |
| ANT. ORG. | 0·03–0·04 × | 0·039 × | 0·033–0·041 × | 0·027 × | 0·027–0·033 × | 0·03 × |
| | 0·02–0·04 | 0·029 | 0·030–0·033 | 0·026 | 0·02–0·027 | 0·025 |
| V.S. | 0·02 × | 0·02 × | 0·026–0·030 | 0·026 | 0·02–0·03 × | 0·028 × |
| | 0·02 | 0·02 | | | 0·02 | 0·02 |

*Sporocyst*

In the digestive gland of mollusc host. Difficult to separate. Elongate and filiform. Active, tapering anteriorly and rounded posteriorly. Subterminal birth pore.

*Emergence and behaviour*

Emergence occurs in the early afternoon and sometimes overnight, and the cercariae tend to aggregate in the lower levels of the water. The resting position

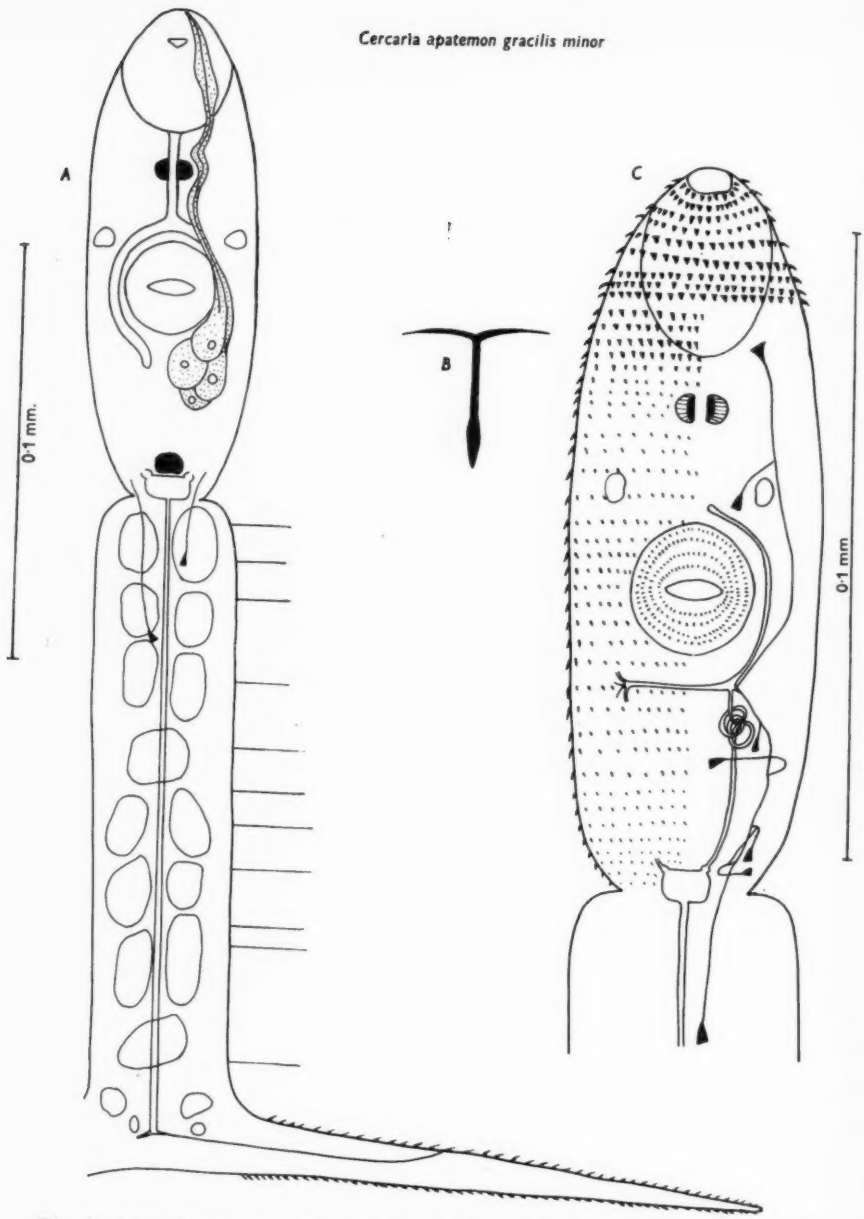


**Fig. 4.** *Cercaria apatemon gracilis minor*. *A*, General structure; *B*, resting position; *C*, spination and excretory system.

in which the body and tail stem hang directly downward with the furcae widely spread, is identical with that of *C. tetraglandis*. It is interesting to note that both species of cercariae penetrate into fresh-water leeches and develop into tetracotyle metacercariae. Both species are negatively phototropic which would facilitate encounter with the secondary intermediate host.

*Host*

*Lymnaea pereger*.

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Small, pharyngeal, longifurcate distome furcocercaria. Four pairs of posterior penetration-gland cells. Flame-cell formula $2(2+4+(1)) = 14$. Posterior excretory commissure and anterior blind ending ducts. Unpigmented eye spots. No penetration spines. Furcae spinose, tail stem aspinose. Eleven pairs of hair-like projections on tail stem and one pair on body. Resting position in which furcae are spread horizontally and body and tail stem hang directly downward.

*Further development*

The cercariae penetrate into fresh-water hirudineans and develop into tetracotyle metacercariae which when fed to laboratory-reared ducklings gave rise to adults of *C. apatemon gracilis minor*, Yam. The adult was described previous to the cercaria, which according to the law of priority becomes *C. a. g. minor*. Observations were made on the specificity and development of the metacercariae and details will be given in a subsequent paper.

*Comparison with related species*

Nine species of cercariae have previously been described with a flame-cell formula and penetration-gland cell number and arrangement identical with that of *C. a. g. minor*. They are: *C. pseudoburti* (Rankin, 1939); *C. burti* (Cort & Brooks, 1928); *C. pygocytophora* (Brown, 1931); *C. okobojensis* (Brooks, 1943); *C. icnusae* (Giovannola, 1937); *C. lessoni* (Johnston & Beckwith, 1947); *C. wansoni* (Fain, 1953); *C. helvetica* XXXI (Dubois, 1929); and *C. a. g. gracilis* (Szidat, 1929*a*, 1931). All these species may be distinguished from *C. a. g. minor* on the following data:

*C. wansoni* is very similar to *C. a. g. minor* in that it possesses both a posterior excretory commissure and anterior blind ending ducts but it may be distinguished on differences in the relative proportions of the body, tail stem and furcae, the presence of papillae on the anterior tip of the body, the number and arrangement of the caudal bodies and its resting position. *C. pygocytophora* differs in the absence of a posterior excretory commissure and the presence of only four pairs of tail stem hairs. *C. pseudoburti*, *C. icnusae*, *C. burti*, *C. okobojensis* and *C. a. g. gracilis* differ from *C. a. g. minor*, in the absence of anterior blind ending ducts, although they possess a posterior excretory commissure. *C. lessoni* possesses a complete anterior

and posterior commissure, although some variation in the completeness of both has been noted, and further differs from *C. a. g. minor* in the more anterior position of the penetration-gland cells.

*C. helvetica* XXXI similarly possesses complete anterior and posterior commissures and may also be distinguished from *C. a. g. minor* on the anterior bifurcation of the oesophagus and the presence of only three rows of spines on the ventral sucker.

These nine species together with *C. a. g. minor* form a very closely related group. The complete life cycles of only three species are known; *C. burti* gives rise to *A. g. burti* (Stunkard, Willey & Rabinowitz, 1941); and the metacercariae are found in fresh-water leeches and are intermediate in structure between a tetracotyle and diplostomulum; *C. a. g. gracilis* develops into a typical tetracotyle metacercaria also in fresh-water hirudineans and gives rise to *A. g. gracilis* Dubois. *C. lessoni* also gives rise to a tetracotyle within fresh-water leeches and develops into *A. intermedius* Johnston. In this connexion it is interesting to note that another subspecies of *A. gracilis*, *A. g. congolensis*, has recently been reported by Dubois & Fain (1956) from the locality in which *C. wansoni* has been found.

### Cercaria X

This species has been well described by Rees (1932) and Erasmus (1954).

*Host*

*Lymnaea stagnalis.*

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Pharyngeal, longifurcate, distome furcocercaria. Four posterior penetration-gland cells. Flame-cell formula $2(3+4+(2)) = 18$. Resting position identical with that of *C. paracauda*.

This last fact is of interest in that the two species are morphologically alike but differ in the flame-cell formula and size. This biological and morphological similarity may be correlated with the fact that both develop into diplostomuli within the lens.

### B. PHARYNGEAL LONGIFURCATE MONOSTOME CERCARIAE

The Vivax *s.l.* subgroup has been subdivided by Szidat (1933), Faust (1920, 1922), Sewell (1922) and Goodman (1951) into the *Vivax* s.str., Tetis, Vivipara and Leptoderma subgroups.

A new species belonging to the Vivax subgroup was found during this study but, owing to its low incidence, an accurate determination of the flame-cell formula and a more detailed investigation of the spination and penetration-gland cell system could not be made.

### (5) **Cercaria vivacis** n.sp. (Fig. 5)

*Morphology*

Body length greater than that of tail stem and furca. Structure of anterior organ more like that of true sucker than penetration organ. Ventral sucker rudimentary. Two sets of penetration-gland cells present posterior and lateral to the pharynx. Subterminal mouth, short prepharynx, large, muscular pharynx, very short oesophagus bifurcating into two large, conspicuous, intestinal caeca which extend to immediately before excretory bladder. Tripartite excretory bladder, lateral vesicles smaller than median chamber. Main excretory canals arising antero-laterally, exhibiting several convolutions in mid-region of body before dividing into anterior and posterior secondary tubules and a transverse canal. The latter joining with its fellow of the other side and with the median tubule formed by the union of two canals arising from the mid-portion of the excretory vesicle. Median caudal canal bifurcating distally, a canal passing into each furca and opening at the tip. Six flame cells in connexion with anterior tubule but only three with posterior tubule. No flame cells observed in the tail stem. Eyespots absent. Genital rudiment represented by rhomboidal mass anterior to excretory bladder, and between distal ends of caeca. Tail stem muscular. Caudal bodies absent. Fin folds on distal half of furcae.

*Spination*

Two rows of penetration spines anterior to the mouth. Aspinose circumoral patch, remainder of body surface spinose. Tail stem aspinose. Furcae bear long hair-like spines even in region of fin folds.

*Measurements* (mm.)

| | Fixed | |
|---|---|---|
| | Range | Average |
| B.L. | 0·13–0·15 | 0·144 |
| B.B. | 0·06–0·09 | 0·073 |
| T.S.L. | 0·09–0·11 | 0·102 |
| T.S.B. | 0·03–0·035 | 0·031 |
| F.L. | 0·08–0·10 | 0·09 |
| F.B. | 0·02 | 0·02 |
| O.S. | 0·03 × 0·03 | 0·03 × 0·03 |

*Sporocyst*

In digestive gland of mollusc host and difficult to separate from host tissue. Posterior end rounded, tapering anteriorly, elongate, filiform, and thin-walled, with solid mass at extremities. Birth pore antero-lateral. Three fixed portions of daughter sporocysts varied in length between 0·85 and 1·15 mm. and in breadth between 0·16 and 0·19 mm.

*Emergence and behaviour*

The cercariae do not emerge in particularly large swarms. Bursts of activity alternate with resting phases in which the furcae are held horizontally and the

proximal portion of the tail stem is bent so that the body is held at an acute angle to the remainder of the tail stem. A characteristic feature of this species is the spoon-shaped curvature of the body apparent during movement and at rest.

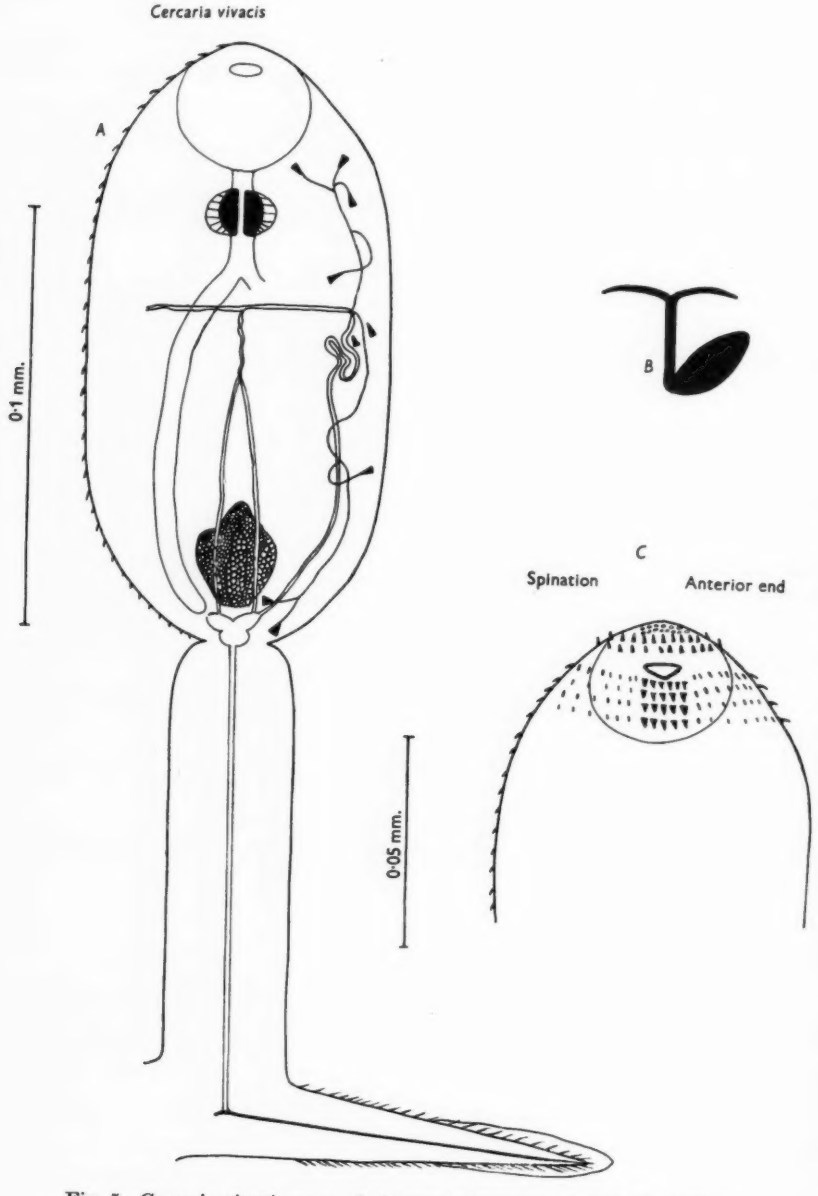


Fig. 5. *Cercaria vivacis* n.sp. *A*, General structure; *B*, resting position; *C*, spination of anterior end.

*Host*

*Bithynia tentaculata* L.

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Pharyngeal, longifurcate, monostome cercaria. Excretory system of 'Vivax' type. Only nine pairs of flame cells observed in body. Fin folds on distal portion of furcae only. Two rows of penetration spines. Body spinose. Tail stem aspinose. Furcae spinose.

*Further development*

Unknown.

*Comparison with related species*

This cercaria is closely related to the members of the 'Vivax' subgroup which are characterized by a rudimentary ventral sucker, the presence of furcal fin folds, the structure of the excretory system and the flame-cell formula $2(12+(3)) = 30$. The species previously described belonging to this subgroup are: *C. vivax* Sonsino, 1892 syn. *C. prohemistomum vivax* (Azim, 1933); *C. vivax* Sonsino, 1894 syn. *C. szidatia joyeuxi* (Joyeux & Baer, 1941); *C. indicae* XV and *C. indicae* LVII (Sewell, 1922); *C. dorsocauda* (Tubangui, 1928); *C. vivax* (Wesenberg-Lund, 1934); *C. kentuckiensis* (Cable, 1935); *C. tatei* (Johnston & Angel, 1940); *C. prohemistomum expeditum* (Balozet, 1953).

*C. indicae* XVIII, *C. p. vivax*, *C. dorsocauda*, *C. tatei* and *C. vivax* (Wesenberg-Lund) may all be differentiated from *C. vivacis* on their larger size and the presence of fin folds along the entire length of the furcae. *C. vivax* Sonsino as described by Wesenberg-Lund differs from that described by Azim in the much shorter tail stem and the aspinose nature, apart from an oral cap of spines, of the body. *C. p. vivax* (Azim) may be further differentiated from *C. vivacis* on the presence of a small ventral sucker. *C. vivax* Wesenberg-Lund and *C. indicae* XV also differ from *C. vivacis* in the aspinose nature of the body. *C. tatei* and *C. indicae* XV may be distinguished from *C. vivacis* also on the presence of excretory concretions in the main excretory canals. *C. indicae* XV is also distinct in that miracidia have been observed to develop within the sporocysts. *C. p. expeditum* differs from *C. vivacis* in the extent of the furcal fin folds and the opening of the furcal excretory tubules midway along the margin of the furcae. *C. kentuckiensis* is also a larger species, but has been too inadequately described to allow a detailed comparison of spination or even of the extent of the furcal fin folds. *C. szidatia joyeuxi* has been inadequately described but may be distinguished from *C. vivacis* on the unusual structure of the excretory system, although it similarly has fin-folds only on the distal part of the furcae. Although only an incomplete description of *C. vivacis* was obtained it is, however, sufficient to distinguish it from those species which have been previously described and it is therefore named as a new species.

*C. kasenyi* (Fain, 1953) differs from the members of the *Vivax* (s.str.) subgroup in the structure of its excretory system but it is similar to *C. vivacis* in possessing fin folds only on the distal portions of the furcae but has fewer flame cells, and differs in the relative lengths of the body, tail stem and furcae.

*Apharyngeal brevifurcate distome cercariae*

Two representatives of this group were studied during this survey, *C. pseudocellata* (Szidat, 1942) and *C. Bilharziellae polonicae* Kowalewski (Szidat, 1929*b*). Szidat (1942) added substantially to the knowledge of this group. He described several new species and showed that the name *C. ocellata* la Valette had been applied to several distinct species which he distinguished on their behaviour and the measurements of the cercariae killed by heat or dilute formalin.

### Cercaria pseudocellata Szidat

Syn. *C. elvae* (Miller, 1926; Matheson, 1930)
*C. ocellata* (Vogel, 1930)
*C. ocellata* (Taylor & Baylis, 1931; Rees, 1932)

Rees (1932) described this species as *C. ocellata* La Valette from the same host and locality. *C. ocellata* La Valette (s.str.) has been described by Dubois (1929) from Neuchâtel and is distinguished from *C. pseudocellata* by its smaller size and its tendency to aggregate near the surface of the water. *C. pseudocellata* tends to adhere by its ventral sucker to the side of the vessel nearest the light source, with its anterior end and tail stem directed outward from the point of attachment.

Rees gave measurements of living specimens only and a comparative table of measurements of *C. pseudocellata* obtained by Rees and the writer is given below:

| | Measurements (mm.) | | | | |
|---|---|---|---|---|---|
| | | Author | | | |
| | | Living | | Fixed | |
| | Rees, Living | Range | Average | Range | Average |
| B.L. | 0·4 | 0·21–0·35 | 0·303 | 0·22–0·286 | 0·261 |
| B.B. | 0·05 | 0·06–0·11 | 0·09 | 0·044–0·066 | 0·055 |
| T.S.L. | 0·42–0·5 | 0·31–0·47 | 0·405 | 0·374–0·454 | 0·424 |
| T.S.B. | ? | 0·04–0·06 | 0·049 | 0·044–0·055 | 0·052 |
| F.L. | 0·32 | 0·27–0·36 | 0·313 | 0·209–0·275 | 0·022 |
| F.B. | ? | 0·03–0·04 | 0·032 | — | 0·022 |
| ANT. ORG. | ? | 0·08–0·11 × 0·05–0·06 | 0·095 × 0·059 | 0·077–0·099 × 0·044–0·044 | 0·093 × 0·044 |
| V.S. | 0·033 | 0·03–0·04 × 0·03–0·04 | 0·039 × 0·038 | — — | 0·022 × 0·022 |
| Eyespots | 0·008 | — | | — | 0·0082 × 0·0005 |
| Distance between eyespots | | 0·03–0·04 | — | 0·0165–0·022 | 0·02 |

*Specific diagnosis*

Apharyngeal, brevifurcate, distome furcocercaria. Five penetration-gland cells, two pairs circum-acetabular and three pairs post-acetabular. Excretory formula $2(3+3+(1)) = 14$. Attaches to the lighted side of the vessel by the ventral sucker with the remainder of the body and tail stem directed outward from the point of attachment.

This species is the causative organism of the schistosome dermatitis incurred by bathers in Roath Park Lake (Taylor & Baylis, 1931).

### (6) **Cercaria bilharziellae polonicae** Kow. (Szidat, 1929*b*) (Fig. 6)

Syn. *C. ocellata* (Ercolani, 1881)

This species has not formerly been reported from Britain but it has been recorded by Wesenberg-Lund (1934) from Europe. Although this species has also been described by Brumpt (1931) and Porter (1938) no details of spination have been given nor have ciliated patches before been observed in the main collecting tubules. Szidat (1929*b*, 1942) observed six pairs of penetration-gland cells in non-emerged cercariae and maintained that the sixth and most anterior pair served in facilitating escape from the mollusc host and were not present in emerged cercariae. Wesenberg-Lund and Porter described six pairs of penetration-gland cells but the former author illustrated only five pairs. Wesenberg-Lund also suggested that a posterior excretory commissure might be present, but this is not substantiated by my observations. The following description is more detailed than that of previous authors and is based entirely on the specimens obtained during this study.

*Morphology*

Short, stout body. Tail stem wider at base with proximal cup-shaped depression. Anterior penetration organ differentiated into proximal glandular and distal muscular portion. Ventral sucker small with H-shaped lumen. Gut rudimentary, comprising simple tube from mouth to anterior margin of ventral sucker, dividing into very short, wide caeca. Small excretory bladder. Main excretory tubules showing convolutions posterior to ventral sucker. Three ciliary patches distinguished in this region on each side. Three anterior flame cells, four posterior, three of which lie in the body and one in the tail stem. Caudal flame cells asymmetrically placed in proximal portion of tail stem. No excretory commissures. Furcal tubules opening at the tip. Furcae very short, with conspicuous fin-folds extending along their entire length. Two pigmented eye spots. Genital rudiment represented by rounded mass of nuclei anterior to excretory bladder and between most posterior pair of penetration gland cells. Five pairs of penetration gland cells present in emerged cercariae.

*Spination*

No penetration spines. Bare circumoral patch. Anterior organ bearing twenty-five to thirty rows of conspicuous closely set spines. Those spines on remainder of body more widely spaced and inconspicuous. Six pairs of short, delicate, hair-like

projections more easily visible in fixed specimens situated anterior to ventral sucker on lateral margins of body. Tail stem and furcae spinose. Numerous small spines on ventral sucker.

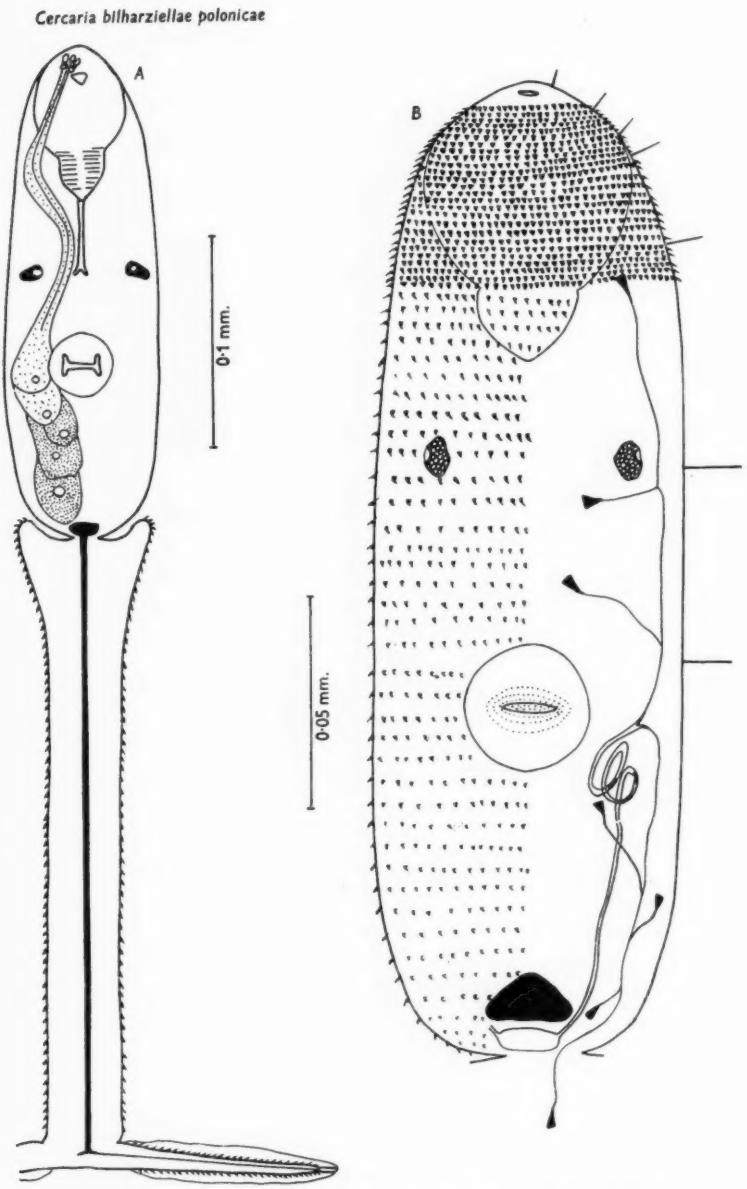


Fig. 6. *Cercaria bilharziellae polonicae*. *A*, General structure; *B*, spination and excretory system.

*Measurements* (mm.)

| | Living | | Fixed | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| B.L. | 0·27–0·36 | 0·31 | 0·21–0·25 | 0·235 |
| B.B. | 0·09–0·12 | 0·105 | 0·07–0·08 | 0·073 |
| T.S.L. | 0·225–0·315 | 0·28 | 0·26–0·30 | 0·291 |
| T.S.B. | 0·053–0·07 | 0·062 | 0·03–0·04 | 0·038 |
| F.L. | 0·105–0·135 | 0·123 | 0·11–0·12 | 0·117 |
| F.B. | 0·01 | 0·010 | 0·01 | 0·01 |
| ANT. ORG. | 0·075–0·09 ×<br>0·06–0·075 | 0·08 ×<br>0·061 | 0·06–0·07 ×<br>0·04–0·05 | 0·067 ×<br>0·047 |
| V.S. | 0·03 ×<br>0·03 | 0·03 ×<br>0·03 | 0·03 ×<br>0·03 | 0·03 ×<br>0·03 |
| Distance between eyespots | | | 0·03–0·04 | 0·034 |

*Sporocyst*

Long, filiform, located in digestive gland of mollusc host. Difficult to separate from host tissue. Terminal birth pore. Numerous constrictions. In living specimens breadth varying between 0·04 and 0·9 mm.

*Emergence and behaviour*

The cercariae emerge in large numbers between 5·00 and 6·00 p.m. They are feeble swimmers and tend to aggregate at the surface of the water where they become entangled in mucus which, according to Szidat (1929*b*), is secreted by the cercariae. Moribund tails and numerous active cercarial bodies are found within the mucous film. It is very difficult to separate the cercariae from the mucus, and degeneration occurs readily once they have become entangled. However, soon after emergence the cercariae will, when disturbed, detach themselves from the mucous film and swim in the upper levels of the water.

*Host*

*Planorbis corneus.*

*Locality*

Roath Park Lake, Cardiff.

*Specific diagnosis*

Apharyngeal, brevifurcous, distome, ocellate, furcocercous cercaria. Five pairs of penetration-gland cells, two pairs anterior and three posterior to ventral sucker. Flame-cell formula $2(3+3+(1)) = 14$. Short, stout body. Base of tail stem expanded with cup-shaped depression. Cercariae tend to aggregate in mucous film at the surface of the water.

*Comparison with related species*

This species belongs to the 'Elvae' group of cercariae (Miller, 1926) but differs from the other members in behaviour, the shorter, stouter nature of the body and the characteristic tail stem with an expanded base and a cup-shaped depression into which the body fits.

*Further development*

This cercaria is known to penetrate directly into the final host, a water fowl, and to develop into the mature trematode within the portal and intestinal blood vessels.

It is interesting to note that both Szidat and Wesenberg-Lund found this cercaria to be relatively rare. In all known occurrences of this species in Europe the mollusc host is *Planorbis corneus* which would seem to indicate a relatively rigid host specificity in the micracidial stage. In Africa, the species recorded as *C. bilharziellae polonicae* by Porter (1938) develops within *Physopsis africana* Krauss and *Planorbis pfeifferi* Krauss, and further differs from my specimens in spination, the position of the posterior flame cells and the presence of six pairs of penetration gland cells; this may be due to the examination of immature non-emerged forms by this author or may indicate that it is a distinct form.

## SUMMARY

(1) Eight species of furcocercous cercariae have been found during a 3-year study of the trematode larvae harboured by certain fresh-water molluscs in Glamorganshire. Of these four are new species, two have previously been reported from South Wales by Rees (1932) and the remaining two species have been recorded in Europe but not in Britain.

(2) Detailed descriptions are given of the four new species and the last two species which had not been completely described hitherto. The life cycles, as far as are known, of all the the species are outlined; the mollusc hosts are listed and for each cercaria a comparison with related species is given.

(3) *C. hamburgensis* Komiya has been proved experimentally to be the larva of *Apatemon gracilis minor* Yam. and according to the law of priority becomes *C. a.g. minor*.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| B.L. | body length |
| B.B. | body breadth in region of ventral sucker |
| T.S.L. | tail stem length |
| T.S.B. | tail stem breadth |
| F.L. | furcal length |
| F.B. | furcal breadth at base |
| ANT. ORG. | anterior penetration organ |
| O.S. | oral sucker |
| V.S. | ventral sucker |

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# STUDIES ON BLOOD GROUP SUBSTANCES ASSOCIATED WITH *ASCARIS LUMBRICOIDES*

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(With 2 Figures in the Text)

## INTRODUCTION

Oliver-González (1946*a*, *b*, 1949) and Oliver-Gonzáles & Torregrosa (1944) have reported upon the existence of substances closely resembling the blood group substances of man and rabbits in polysaccharide preparations from parasitic helminths such as *Ascaris lumbricoides*, *Trichinella spiralis*, *Fasciola hepatica*, *Taenia saginata*, *Necator americanus*, *Schistosoma mansoni* and *Cysticercus cellulosae*. Such materials neutralized anti-A agglutinins and some also neutralized the anti-B agglutinins in human serum. When living worms or larval stages of *Schistosoma mansoni* and *Trichinella spiralis* were incubated in human anti-A serum complete neutralization of the $\alpha$-isoagglutinins occurred, indicating that the specific neutralizing substance in these parasites is produced or associated with the living worm and does not have to be uncovered by chemical processes employed in the preparation of the polysaccharides. These living worms were not able to affect the B antibody levels. When rabbits, lacking the group A substance in their tissues, were infected with *Ascaris lumbricoides* a marked increase in anti-A agglutinins occurred but there was no significant increase in anti-B agglutinins.

Recently Cameron & Staveley (1957) have demonstrated the blood group P substance in the fluid of fertile hydatid (Echinococcal) cysts and they suggest that this substance is associated with the germinal layer of the cyst.

As far as can be ascertained no reports exist of the effect of such helminth infections on the iso-antibody levels in the natural host for the parasites. This report deals principally with the effect of *A. lumbricoides* infection on the A antibody levels in pigs but also includes observations on the effect of *Oesophagostomum dentatum* infection on such antibodies.

## MATERIALS AND METHODS

### *Animals*

Three groups of pigs were used. The first group of 7 (group 1) was obtained from a reputed *Ascaris lumbricoides*-free herd. Since, later, some doubt existed as to the validity of the claim to freedom from ascaris, all further pigs were obtained by Caesarian section and were reared in sterilized cages for one month, being fed on an artificial diet and deprived of colostrum, afterwards being kept in individual pens. The first group of pigs (group 1) was infected with *A. lumbricoides* eggs at 12 weeks of age. A second group of five artificially reared pigs (group 2) was infected

at 17 weeks of age and a third group of five comparable pigs (group 3) was infected with *Oesophagostomum dentatum* third-stage larvae at 7 weeks of age.

Blood was obtained from ear veins and serum was collected after overnight separation. It was stored at −20° C. and inactivated at 56° C. for 30 min. before use.

### *Parasite materials*

*A. lumbricoides* eggs were obtained from the terminal portion of the uterus of fresh worms and cultured in 1 % formalin at 27° C. for 40 days.

Ascaris larvae were obtained from the lungs of rabbits 7 days after they had received a large dose of eggs. Larvae were separated from lung tissue by the Baermann technique. Larval 'metabolic products' consisted of the supernatant fluid of a Stoll Ringer suspension of larvae kept at 37° C.

An extract of adult worms was made by macerating fresh worms in a high-speed emulsifier (Atomix), centrifuging the resulting material and retaining the supernatant. Separate extracts were made from worms obtained from blood groups O and A pigs.

### *Serological tests*

The A–O grouping of pigs was established by means of the 'naturally occurring' anti-A in group O pigs. The serological grouping, as well as the titration of anti-A in pig sera, was performed by means of the antiglobulin sensitization test using a rabbit anti-pig globulin serum (Saison, Goodwin & Coombs, 1955).

Demonstration of A and B substances in ascaris extracts was by simple inhibition tests.

## RESULTS

### *Neutralization of human and pig anti-A by ascaris extracts*

Four extracts of whole ascaris worms (see methods) made from worms collected from two group O pigs and two group A pigs were capable, even at a 1/20 dilution, of inhibiting 100 sensitizing doses of pig anti-A. Human anti-A (and anti-B) were similarly inhibited. On the other hand, the extracts showed not the slightest inhibition of human anti-D Rh agglutinin.

That the 'metabolic products' of living ascaris larvae contain the A substance was shown by testing the 'culture fluid' containing the larvae at varying intervals after larvae were introduced. Small volumes of fluid were removed at intervals and titrated for their ability to inhibit the sensitization of pig A red cells by 10 doses of the A iso-antibody (Goodwin & Coombs, 1956). At zero hour the culture fluid had no inhibiting power, at 16 hr. the culture fluid inhibited 10 doses of A iso-antibody at a dilution of 1/256 and at 48 hr. a 1/512 dilution of culture fluid inhibited this amount of iso-antibody.

### *Ascaris infection in pigs of blood groups A and O*

Since adult and larval ascaris materials contained A substance it was of interest to know whether the A–O blood group of a pig affected its ability to become infected, since a group O pig with A antibodies might be infected with less facility than a group A pig. Blood samples were collected and the presence of ascaris

determined on a series of pigs at a bacon factory. Of ten pigs infected with adult worms, four were blood group A and six were blood group O, thus indicating that the possession of a specific blood group did not prevent infection with *A. lumbricoides*.

*The effect of* Ascaris lumbricoides *infection on the level of anti-A in pigs*

Of the first group of seven pigs (group 1) two (8, 10) were each infected with 300,000 *A. lumbricoides* eggs as a single dose, three (4, 6, 9) each received 1000 eggs daily for 5 days, the remaining two pigs (3, 7) serving as controls. One month after the initial infection, one control pig (3) and two of the group which had received 1000 eggs daily for 5 days (10, 4) were each given a further single dose of 500,000 eggs. The resulting A antibody levels are illustrated in Fig. 1. Control pig 7 was later found to be infected and since it was housed with an infected pig (4) it was probably infected either by eggs which were excreted following dosing, or from eggs resulting from the patency of the infection in pig 4. Similarly, control pig 3 was found to be naturally infected. Pig 10 is excluded from the results since it possessed blood group A and, as would be expected, no anti-A levels were detected. The results indicate that there is a pronounced rise in A antibody levels in pigs following infection with *A. lumbricoides*. Though the experiments are somewhat unsatisfactory because of the probability of natural infection and also cross-infection, there can be little doubt that the marked responses seen were due to infection with the parasite.

The second group of five pigs (group 2) was reared to ensure freedom from both ascaris and maternal antibody, and of these one pig (19) received a single dose of 500,000 eggs, two (18, 20) each received 1000 eggs daily for 5 days and the remaining two pigs (16, 17) served as controls. The resulting A antibody levels are illustrated in Fig. 2. This group shows more clearly the relationship between ascaris infection and iso-antibody levels.

*Effect of* Oesophagostomum dentatum *infection on A antibody levels in pigs*

Of a further group of five artificially reared pigs (group 3) three were infected with 150,000 third-stage *O. dentatum* larvae at 7 weeks of age, two pigs serving as controls. This infection did not induce any A antibody formation, all serum samples being negative for a period of 8 weeks after infection.

## DISCUSSION

This work confirms the findings of Oliver-González & Torregrosa (1944) that human anti-A and anti-B agglutinins are neutralized by material from *Ascaris lumbricoides*. The present work has shown that the A-like substance in *A. lumbricoides* also reacts with, and inhibits, the anti-A in pig sera and that infection stimulates the production of anti-A in these animals.

The presence of A substance in the metabolic products of ascaris larvae and in larval tissues is of particular interest since it indicates that this substance is excreted and/or secreted by ascaris larvae and will be associated with larvae migrating in the host. The view is generally held that such metabolic products of

nematodes play an important part in inducing immunity and the reaction of antibodies with such metabolic products is considered to be an essential process in halting growth and development of nematodes in an immune animal (Taliaferro

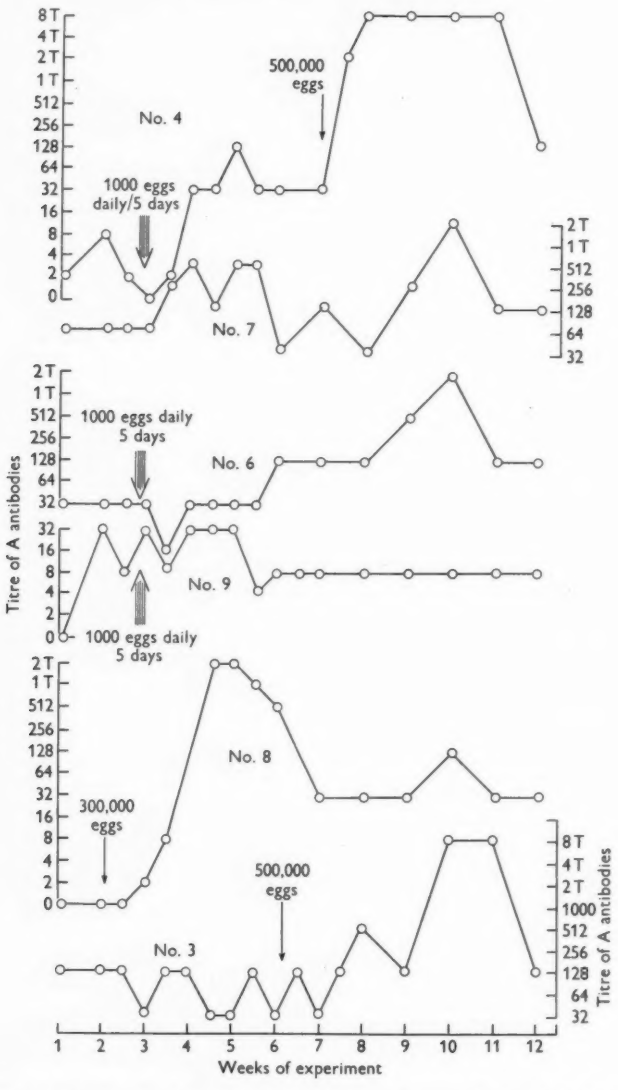


Fig. 1. The levels of A antibody in six pigs of group 1 after infection with *Ascaris lumbricoides* eggs.

& Sarles, 1939). Since group O pigs may become infected with *A. lumbricoides* it would appear that at least the A antibodies are unable to cause a detrimental effect on the worm. It is probable that the A substance in metabolic products and

elsewhere is a 'non-essential' antigen as far as protective immunity is concerned and that its presence in metabolic products is fortuitous.

It is interesting to record the stimulation of anti-A levels in pigs by *A. lumbricoides* infection and the inability of *Oesophagostomum dentatum* infections to do this. Since probably the majority of pigs kept in commercial herds are, or have been, infected with ascaris to a varying degree, this parasite may be in part responsible for the marked variations in anti-A levels which are seen and have been commented upon by Goodwin & Coombs (1956).

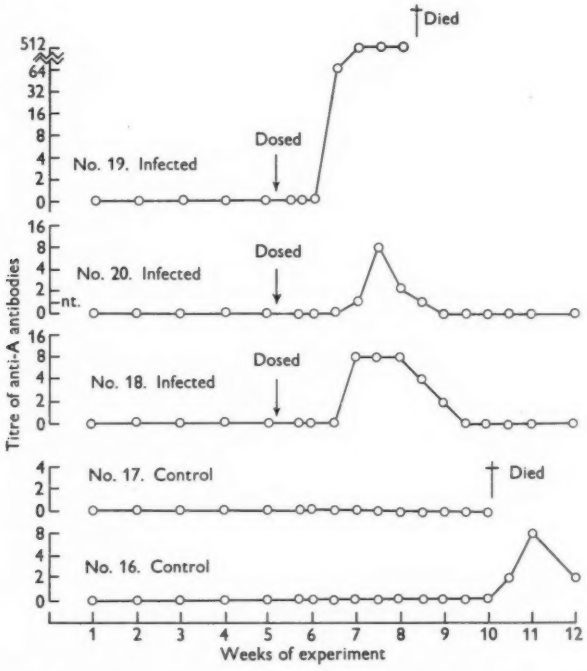


Fig. 2. The levels of A antibody in five pigs of group 2 after infection with *Ascaris lumbricoides* eggs. No. 19 received a single dose of 500,000 eggs, whilst nos. 18 and 20 each received 1000 eggs daily for 5 days. Nos. 16 and 17 were not infected.

It would be interesting to know whether the seasonal variations in iso-antibodies in other animals, particularly in cattle, where the anti-J levels show a marked variation (Stone, 1956), are in part due to parasitic infections, especially since it has been shown that A-substance exists in *Fasciola hepatica* (Oliver-González & Torregrosa, 1944), a parasite which commonly affects cattle.

The role played by high induced levels of iso-antibodies in the pathogenesis of parasitic disease is difficult to assess. No clinical evidence exists to suggest that iso-antibodies induced by nematodes are of importance in causing ill-health. From the theoretical point of view Oliver-González (1953) has shown that polysaccharide materials from *A. lumbricoides* and other parasites can be adsorbed on to erythro-

cytes. He suggests that such new erythrocyte-polysaccharide complexes would induce antibody production which would in fact be directed against the host's own erythrocytes. Whilst no evidence exists that this occurs in actual parasitic infections, a further examination of the causes of anaemia associated with some parasitic infections may show that iso-antibodies stimulated by the parasites are of some importance in the causation of pathogenic effects.

## SUMMARY

Human anti-A and anti-B and pig anti-A agglutinins were neutralized by *Ascaris lumbricoides* materials.

The infection of pigs with *A. lumbricoides* stimulated the production of anti-A agglutinin but this agglutinin appeared to have no protective action.

*Oesophagostomum dentatum* infection in pigs did not affect the anti-A agglutinin levels.

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# LOUSE POPULATIONS OF SOME RODENTS OF THE GREAT SALT LAKE DESERT*

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(With 1 Figure in the Text)

## INTRODUCTION

Up to the present there has not been a full season's study of louse populations on wild mammals of the United States. The only known paper dealing with this subject was written by Vysotskaia (1950). In this paper Vysotskaia studied the fluctuations of lice on the grey vole, *Microtus arvalis* (Pallas), in the Leningrad area of U.S.S.R. The present study is an attempt to establish some comparative basis for workers in this area. The study area was the southern arm of the Great Salt Lake Desert of north-western Utah, and included western Tooele, Box Elder and Juab counties.

The rodents used were the Ord kangaroo rat, *Dipodomys ordii pallidus* (Durrant & Setzer); the white tail antelope ground squirrel, *Citellus leucurus leucurus* (Merriam); and the deer mouse, *Peromyscus maniculatus sonoriensis* (LeConte). These rodents were selected because of their availability and because they represented three families of rodents which harbour most of the lice found in this area (Ignoffo, 1956). All animals were live-trapped from either juniper-brush or mixed-brush habitats. The majority of specimens were obtained from the juniper-brush habitat. Flowers (1953), who worked in the same area, gave this description of a juniper-brush habitat:

> The junipers grow...in groups; within these groups the trees are 15 to 100 feet apart and between the groups...open spaces 100 yards or so in diameter...or only dead stumps. The junipers are not uniform in size or age...usually 15 to 20 feet high, slightly less...than across the branch spread...several large trees 30–35 feet tall are scattered among the others....The area contains...this botanical composition...
>
> | | |
> |---|---|
> | Dominant | *Juniperus osteosperma* (Torr.) Little |
> | Frequent | *Sarcobatus vermiculatus* (Hook.) Torr. |
> | | *Atriplex canescens* (Pursh.) Nutt. |
> | | *Tetradymia glabrata* Gray |
> | | *Chrysothamnus viscidiflorus* var. *pumilus* (Nutt.) Jep. |
> | Occasional | *Eriogonum dubium* Stokes |
> | | *Gutierrezia sarothrae* (Pursh.) B. &. S. |
> | | *Aster* spp. |
> | | *Oryzopsis hymenoides* (R. & S.) Richer |
> | | *Bromus tectorum* L. |

In this study rodents were live-trapped in tin-can or Havahart traps and transferred to 5 lb. size cloth flour-sacks for transport back to the laboratory. The

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rodents were then killed without removal from the sacks and placed under refrigeration for approximately 24 hr. The flotation method, Gering & Thomas (1953), was used to obtain lice from the Ord kangaroo rat and the deer mouse. These rodents were processed by William Bacha,* who collected and separated ectoparasites into lice, ticks, mites and fleas for study by subsequent workers. The flotation method followed this procedure: refrigerated animals were first brought to room temperature and then placed into quart jars; a teaspoon of Calgon (sodium hexametaphosphate detergent) was added; each jar was filled with water and agitated for approximately 10 min. Separatory funnels were used to recover ectoparasites for identification and subsequent counting.

The white tail antelope ground squirrels were processed by the brushing technique. A blunt-edged scalpel was used instead of the customary tooth brush. This reduced the possibility of contamination between specimens. Louse population records for each rodent species were maintained from February 1955 to January 1956.

## LOUSE–HOST ASSOCIATIONS

Five species of Anoplura were recovered from three rodent species. Table 1 lists these associations as well as the total and percentage of lice recovered for a 1-year sample.

Table 1. *Lice associated with various rodents found in the southern regions of the Great Salt Lake Desert*

| Rodent species | Lice species | Total lice | |
|---|---|---|---|
| | | No. | % |
| *Citellus leucurus* | *Neohaematopinus citellinus* | 3983 | 88·8 |
| | *Enderleinellus suturalis* | 501 | 11·2 |
| *Dipodomys ordii* | *Fahrenholzia pinnata* | 729 | 100·0 |
| *Peromyscus maniculatus* | *Hoplopleura hesperomydis* | 729 | 96·3 |
| | *Polyplax auricularis* | 28 | 3·7 |

The dominant species of louse on the deer mouse was *Hoplopleura hesperomydis* (Osborn), which made up 96 % of the total population. *Polyplax auricularis* K. & F. accounted for the other 4 %.

Cook & Beer (1955), in their study of louse populations on deer mice in Minnesota, did not recover *P. auricularis* after dissolving a total of 380 skins over a period of 2 years. This louse is primarily restricted to *Peromyscus* hosts, but it has also been recovered from *Onchomys torridus* (Coues) in California, from *O. leucogaster* (Wied) in Colorado and Kansas, from *Reithrodontomys mexicanus* (Saussure) and from *Neotomodon alstoni* Merriam from Mexico (Hopkins, 1949; Ferris, 1951). In the United States, *Polyplax auricularis* has been taken from rodents found only in the western areas. An infestation has been recorded as far east as Kansas. On the other hand, *Hoplopleura hesperomydis* is more continental in distribution. This

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species has been widely reported from *Peromyscus*, *Onychomys*, *Mus*, *Microtus* and *Reithrodontomys* over most of the United States.

Two species of lice, *Neohaematopinus citellinus* Ferris and *Enderleinellus suturalis* (Osborn), were recovered from the white tail antelope ground squirrel. *N. citellinus* accounted for 89 % of the total louse population on ground squirrels, while *E. suturalis* made up the remaining 11 %. *N. citellinus* has been recovered only from ground squirrels in western North America. *E. suturalis* is also primarily western in distribution and has been recovered from *Citellus franklinii* (Sabine) and *C. tridecemlineatus* (Mitchell), as far east as Minnesota (Ignoffo, 1957).

The only species of louse found on Ord kangaroo rats was *Fahrenholzia pinnata* K. & F., which is generally restricted to *Dipodomys* species, but has also been recovered from species of *Perognathus*. Recorded specimens, infested with *F. pinnata*, have been restricted to the areas west of the Rocky Mountains.

## HOST INFESTATION

Average infestations of the three rodents are presented in Table 2. Three different values are utilized. These are per cent infestation, lice per total hosts and lice per infested host.

Table 2. *Average infestation rate of three rodents*

| Host species | Lice species | No. of hosts | % infestation | Lice Host | Lice Inf. host |
|---|---|---|---|---|---|
| *Citellus leucurus* | *Neohaematopinus citellinus* | 174 | 79·2 | 22·9 | 27·7 |
| | *Enderleinellus suturalis* | 139 | 38·9 | 3·4 | 8·6 |
| *Dipodomys ordii* | *Fahrenholzia pinnata* | 154 | 48·7 | 4·7 | 9·6 |
| *Peromyscus maniculatus* | *Hoplopleura hesperomydis* | 192 | 50·0 | 3·8 | 7·6 |
| | *Polyplax auricularis* | 192 | 6·2 | 0·2 | 2·5 |

The rate of infestation of *Hoplopleura hesperomydis* on deer mice for one complete year falls between 0 and 50 %, depending upon whose study is quoted. On the basis of a 2-year study, Cook & Beer (1955) recorded an average infestation of 41·0 and 15·0 from samples of 105 and 275 deer mice. Harkema (1936) did not recover any lice from 54 deer mice which he examined over part of one year. Royal (1952) obtained results similar to Harkema. He found only 4 specimens infested with 5 lice. This was based on a sample of 42 deer mice collected over a period of a year. Morlan & Hoff (1957) examined 518 deer mice over a period extending from July 1951 to February 1954 and found 40 mice infected with *H. hesperomydis*. During the same period they also recorded 5 of 25 piñon mice, *Peromyscus truei*, 1 of 3 brush mice, *P. boylei*, and 1 of 21 rock mice, *P. nasutus*, infested with the same species.

*Polyplax auricularis*, on the other hand, was recovered from only 6·2 % of the 192 deer mice. This agreed quite well with data obtained by Morlan & Hoff (1957), who found 45 of 512 deer mice infested with this louse.

The infestation rate of *Fahrenholzia pinnata* on the Ord kangaroo rat was much higher than that obtained by Morlan & Hoff (1957), who found only 71 of 2232 rats infested with *F. pinnata*.

During 1952 and 1953, respectively, Cook & Beer (1956) obtained values for *Hoplopleura hesperomydis* of 6·4 and 1·0 per deer mouse and 15·0 and 7·8 per infested deer mouse. Morlan (1952) recorded 8·2 *H. hesperomydis* per infested cotton mouse, *Peromyscus gossypinus*, and 2·9 *Polyplax auricularis* per infested deer mouse. Morlan & Hoff (1957) obtained infestation of 3·3 *H. hesperomydis* and 2·9 *P. auricularis* per specimen after examining 518 deer mice over a period of 31 months.

Infestation values of 4·7 *Fahrenholzia pinnata* per host and 9·6 *F. pinnata* per infested host were obtained from the Ord kangaroo rat. Morlan & Hoff (1957) collected 123 *F. pinnata* from 71 infested kangaroo rats or 1·7 lice per infested specimen.

In the present study the highest individual infestation rate was recorded from the white tail antelope ground squirrel. Values of 22·9 lice per host and 27·7 lice per infested specimen were calculated from squirrels infested with *Neohaematopinus citellinus*. In contrast the infestations of *Enderleinellus suturalis* on the same host were 3·4 and 8·6 respectively. In a similar study of the spotted ground squirrel, *Citellus spilosoma*, Morlan & Hoff (1957) recovered 176 *N. citellinus* from 34 infested specimens and 10 *E. suturalis* from 2 infested hosts.

## FREQUENCY DISTRIBUTION OF LICE ON THE HOSTS

The frequency distribution of lice on the white tail antelope ground squirrel, the deer mouse and the Ord kangaroo rat is depicted in Fig. 1A. The distribution of lice on all three rodents was typical of the general pattern obtained from similar studies. The distributions are skewed by the small number of hosts which harbour large populations of lice. Table 3 presents a breakdown of the total rodent sample into one of three groupings based upon the number of lice found on each rodent.

Table 3. *Distribution of the total rodent sample into various groupings*

(Values are expressed as % of the total sample based upon a 1-year sample.)

| | Rodent hosts | | |
|---|---|---|---|
| No. of lice | *Citellus leucurus* | *Dipodomys ordii* | *Peromyscus maniculatus* |
| 0 | 20·1 | 51·9 | 50·0 |
| 1–25 | 59·1 | 40·9 | 46·9 |
| > 25 | 20·7 | 7·1 | 3·1 |

The louse group 26 and above on deer mice makes up 3 % of the total sample, yet it accounted for 53 % of the total population of *Hoplopleura hesperomydis*. This general relationship also existed on the white tail antelope ground squirrel and the Ord kangaroo rat. Approximately 21 % of the squirrels had 26 or more *Neohaematopinus citellinus* per host, but yet accounted for 82 % of the total population of lice. During the same period 7 % of the Ord kangaroo rats had 26 or more lice per host, which accounted for > 65 % of the total recovered lice. Since 0–25 lice per host represents 80 % of the rodent population sampled, this lice grouping may serve as a better basis for estimating seasonal variation.

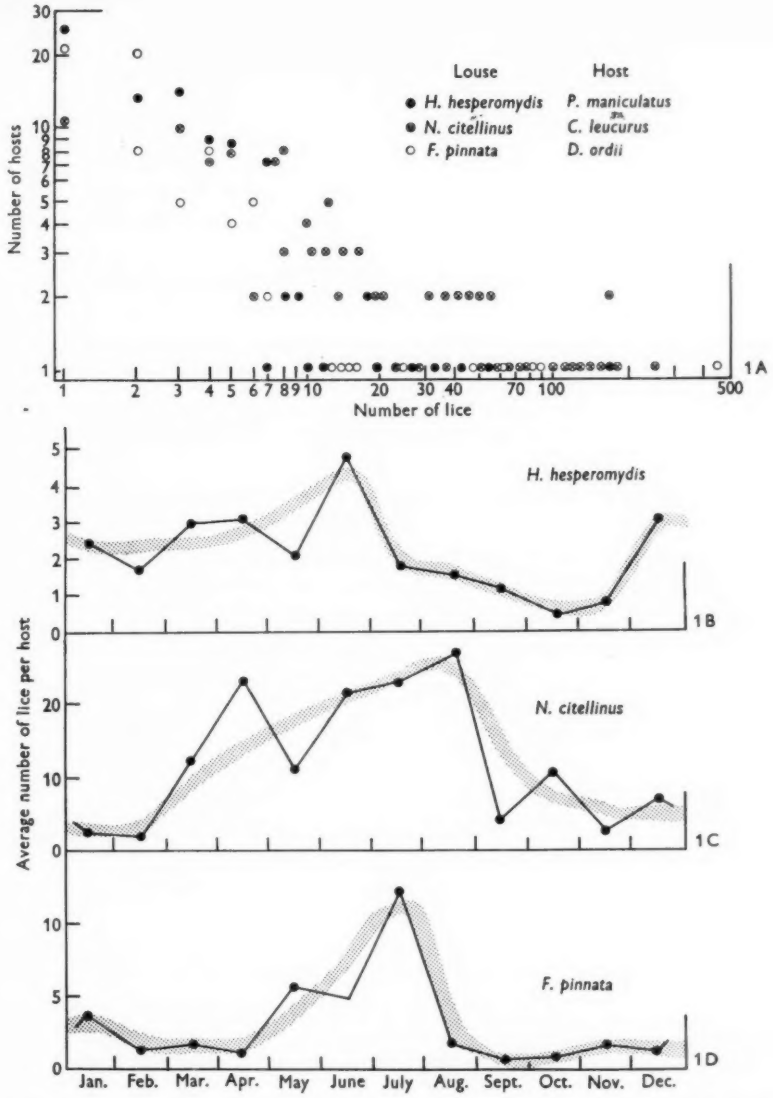


Fig. 1. Frequency distribution and seasonal fluctuations of lice populations of various rodents. A, frequency distribution of lice on *Peromyscus maniculatus sonoriensis* (LeConte), *Citellus leucurus leucurus* (Merriam), and *Dipodomys ordii pallidus* Durrant & Setzer; B, seasonal change of *Hoplopleura hesperomydis* (Osborn) on *Peromyscus maniculatus sonoriensis* (LeConte); C, seasonal change of *Neohaematopinus citellinus* Ferris on *Citellus leucurus leucurus* (Merriam); D seasonal change of *Fahrenholzia pinnata* K. & F. on *Dipodomys ordii pallidus* (Durrant & Setzer); the shaded portion represents a line drawn free-hand on the graph.

## SEASONAL LOUSE POPULATIONS

What technique would best show the seasonal variation of lice? This has always been a perplexing question. Previously average values of ectoparasites per specimen per month were used. Values in these instances are influenced by the small number of hosts which harbour large louse populations. To reduce this influence Rumreich (1945), Cole & Koepke (1946), and others (Biometric Society 1948) suggested using the technique of transformed data. In this study individual samples within each month were subjected to a transformation of $\sqrt{(x+0{\cdot}5)}$ to obtain a monthly mean. This mean was then untransformed and plotted as an average value of lice per host for each month. The results of the dominant louse species are presented in Fig. 1B for white-footed deer mice, Fig. 1C for white tail antelope ground squirrels, and Fig. 1D for Ord kangaroo rats.

Two peaks were recorded for populations of *Hoplopleura hesperomydis* on deer mice. The major peak occurred in June, with a minor peak occurring in December. Low population levels were recovered during the summer and early fall season, the lowest point being recorded in October. Fluctuations recorded from January to May are probably not significant and may reflect individual variation within each monthly sample.

An August peak was recorded for *Neohaematopinus citellinus* on white tail antelope ground squirrels. Variations between the months of April and May are probably not significant. Low populations of *N. citellinus* occurred from September to February. High populations of *N. citellinus* were present from March to April.

The major population increase for *Fahrenholzia pinnata* on Ord kangaroo rats began during April and reached its peak in July. After July the mean population size decreased rapidly to a September low. In general the population of *F. pinnata* was maintained at a low level from August to the following May.

Correlation coefficients for each host used in this study were calculated for temperature and precipitation as against lice per host per month. In addition, host-activity and host-density were compared with lice per host per month for both deer mice and Ord kangaroo rats. Data for activity and density were obtained from Rosasco (1955), who studied the seasonal population and seasonal activity of these two rodents from the same area in which the present louse-population study was conducted. Temperature and precipitation records are those of the Salt Lake City area and represent a 20-year average. Of the ten correlations that were calculated, two showed significance within the 5% level. The relationship between activity of deer mice and population of *Hoplopleura hesperomydis* showed a negative correlation at the 2% level of significance. On the other hand, when temperature was correlated with lice per white tail antelope ground squirrel, a positive coefficient was obtained at the 1% level. A positive coefficient was also obtained between temperature and lice per Ord kangaroo rat, but this was only significant at the 10% level.

SUMMARY

During the period extending from February 1955 to January 1956, 192 specimens of deer mice, *Peromyscus maniculatus sonoriensis* (LeConte), 174 specimens of white tail antelope ground squirrels, *Citellus leucurus leucurus* (Merriam) and 154 specimens of Ord kangaroo rats, *Dipodomys ordii pallidus* (Durrant & Setzer), were examined to determine louse-host associations, rate of infestation, frequency of distribution and seasonal fluctuation of louse populations.

Five species of lice were recovered from three species of rodents. The dominant species on the deer mouse was *Hoplopleura hesperomydis* (Osborn). The dominant species on the white tail antelope ground squirrel was *Neohaematopinus citellinus* Ferris. The only louse species recovered from the Ord kangaroo rat was *Fahrenholzia pinnata* K. & F.

Infestation on rodents was recorded on the basis of percentage of hosts infested, number of lice per host and number of lice per infested host. The highest number of lice per host was 27·7 *Neohaematopinus citellinus* per infested white tail antelope ground squirrel. The lowest value was 2·5 *Polyplax auricularis* per infested deer mouse.

Approximately 80 % of the rodent sample had less than 25 lice per host. This grouping accounted for about one-half of the total number of recovered lice.

Seasonal fluctuations of louse populations on deer mice, white tail antelope ground squirrels and Ord kangaroo rats were studied over one complete year. There appeared to be some coincidence between rodent activity, temperature and louse populations.

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* English translation of this paper was obtained from Dr E. Cook. The original Russian text was translated by Dr M. Haydak.

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# THE LIFE HISTORY AND PATHOGENICITY OF *EIMERIA MELEAGRIDIS* TYZZER, 1927, IN THE TURKEY POULT

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(With 2 Figures in the text)

## I. INTRODUCTION

*Eimeria meleagridis* was the first species to be described from the turkey, by Tyzzer, briefly in 1927 and more completely in 1929. He described the morphology of the oocysts and the sporulation time and prepatent period. The oocysts mainly developed in the caeca, as did large schizonts, which were unique among avian coccidia in that they contained a residual body when the merozoites had formed. The oocysts were not infective to chickens. No further work was carried out until Steward (1947) infected chickens with oocysts from turkeys which were of the same size and shape as *E. meleagridis* and differed from those of *E. meleagrimitis*, the only other turkey species then known. Morehouse (1949*a*) claimed that *E. meleagridis* caused a mortality of 6·3 % and described the control of coccidiosis caused by this species with sulphonamides (Morehouse, 1949*b*). When Moore & Brown (1951) described *E. adenoeides* as a very pathogenic new species they suggested that Morehouse may have been dealing with a mixed infection, since the oocysts of *E. meleagridis* and *E. adenoeides* are indistinguishable and since they found that *E. meleagridis* was not, in fact, pathogenic. This may have applied also in the field outbreak of turkey coccidiosis reported by Wilson (1951) in Britain which, he believed, from oocyst measurements, was due to *E. meleagridis* and *E. meleagrimitis*; this was just before *E. adenoeides* was described. Hawkins (1950), whose work was not generally made known until 1952, increased our knowledge of the turkey coccidia enormously. He confirmed Tyzzer's work and described the life cycle, pathology, and pathogenicity of the three species then known, including *E. meleagridis*, which he said was not pathogenic and passed its life cycle in the caecum. Hawkins also described as a new species *E. gallopavonis*, the oocysts of which were indistinguishable from those of *E. meleagridis*. Demke (1952) found that a strain of *E. meleagridis* isolated from a field outbreak of coccidiosis was non-pathogenic under laboratory conditions.

Much of this knowledge was summarized by Moore (1954), who pointed out that the oocysts of *E. meleagridis*, *E. gallopavonis* and *E. adenoeides* are indistinguishable and that species identification cannot therefore be based on this character alone. The present paper reports the isolation of a pure strain of an *Eimeria* from turkeys in Great Britain, describes its life history and pathogenicity and gives reasons for identifying it as *E. meleagridis*. Accounts have already been published of *E. adenoeides* (Clarkson, 1958) and *E. meleagrimitis* (Clarkson, 1959).

## II. MATERIAL AND METHODS

These were the same as in previous papers and are described fully in the study of *E. adenoeides* (Clarkson, 1958). A pure strain was obtained by infection with a single oocyst using coccidia-free poults. The oocyst was selected from material obtained by infecting a poult immune to *E. adenoeides* with sporulated oocysts from the faeces of turkeys collected on a farm and therefore probably harbouring a mixed infection. By this means it was believed that the oocysts in the sample which were *E. adenoeides* would not develop, whereas oocysts of other species would. After sporulation ellipsoidal oocysts were selected by means of a micromanipulator from those produced by the bird immune to *E. adenoeides*.

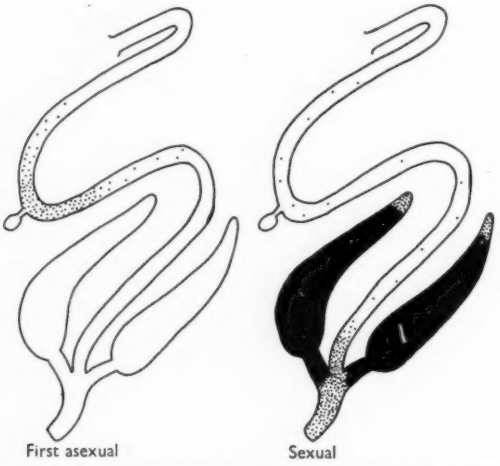


Fig. 1. Localization of first asexual and sexual generations of *E. meleagridis* in the intestine of the poult.

## III. RESULTS

### (*a*) *The life history*

Previous workers have stated that every stage of the life cycle occurs in the caecum, but in a bird which was killed at 54 hr. large numbers of merozoites were found in the small intestine around the rudimentary yolk stalk, whereas a bird of the same series killed at 120 hr. showed larger numbers of oocysts in the caecum and none in the small intestine. After this finding very careful search was made of the whole intestine of every bird which was killed and the results largely confirm the initial findings. The first asexual generation develops only in the epithelial cells of the small intestine, a short distance on each side of the rudimentary yolk stalk. The second asexual generation is found in the caecum, as is the sexual generation, which shows some spread into the rectum and ileum (Fig. 1). In a few birds, oocysts in very small numbers have been found in the small intestine anterior to the rudimentary yolk stalk by scraping the mucosa, but no sexual stages have been seen in tissue sections of this region. The significance of these findings is discussed later.

Small numbers of 2nd-stage schizonts and sexual stages were found in the deep glands of the caecum as well as the surface epithelium. This character has previously been said to be confined to *E. adenoeides* (Moore, 1954).

Sporozoites (Fig. 2A) were obtained from the intestine of a bird given three doses, each of a million sporulated oocysts, at hourly intervals and killed 2 hr. after the last infection. Their movement and morphology were similar to those of the other *Eimeria* species of the turkey (Clarkson, 1958, 1959). 24 hr. after infection rounded trophozoites were found in the epithelial cells of the small intestine, each containing a large 'globule' and a single nucleus (Fig. 2B). Most of the 1st-stage

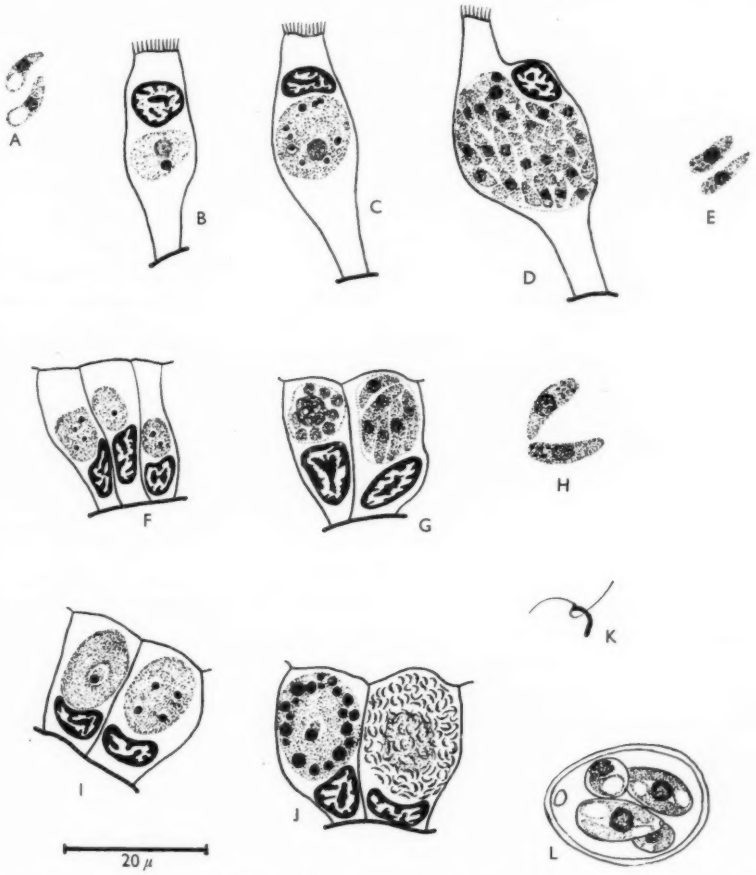


Fig. 2. Life cycle of *E. meleagridis*. A, Sporozoites; B, trophozoite: 12 hr.; C, developing schizont: 36 hr.; D, mature 1st-stage schizont: 48 hr.; E, 1st-stage merozoites: 60 hr.; F, developing 2nd-stage schizonts: 72 hr.; G, mature 2nd-stage schizonts: 84 hr.; H, 2nd-stage merozoites: 84 hr.; I, early gametocytes: 96 hr.; J, macrogamete, microgametocyte showing residual mass and microgametes: 108 hr.; K, microgamete: 108 hr.; L, sporulated oocyst. A, E, H and K, Giemsa-stained smears; L, fresh preparation; remainder: sections stained with Heidenhain's iron haematoxylin (camera lucida, × 1600). The thick line represents the base of the epithelial cells.

schizonts develop near the base of the villi but none has been seen in the deep glands. The schizont develops rapidly and the nuclei divide (Fig. 2C), giving rise to a fully developed schizont measuring $20 \times 15\mu$, lying below the epithelial cell nucleus, and containing 50–100 merozoites (Fig. 2D). These mature schizonts were present as early as 48 hr. after infection and attained a maximum at about 60 hr., but some were retarded in their development and were found as late as 5 days after infection. This may possibly have been due to re-infection, but every endeavour was made to minimize this by transferring the bird to a clean cage daily. 1st-stage merozoites measured $7 \times 1{\cdot}5\mu$ (Fig. 2E).

No stage was seen in the caecum until 52 hr., when uninucleate trophozoites of the second asexual generation were found in one bird, but at 60 hr. many developing schizonts were present (Fig. 2F). Mature schizonts which measured about $9\mu$ in diameter and contained 8–16 merozoites were seen as early as 70 hr. after infection, with a peak at 84 hr. (Fig. 2G). These schizonts developed above the cell nucleus and many possessed a round residual mass. The merozoites were larger than those of the first generation and measured about $10 \times 2\mu$ (Fig. 2H).

In a bird killed at 91 hr. uninucleate stages were found, and by 96 hr. it was obvious that these were developing into gametocytes (Fig. 2I) which were mature by 108 hr. (Fig. 2J), were situated above the cell nucleus and measured about $18 \times 13\mu$. There was a large rounded residual mass in the microgametocyte. The microgametes were biflagellate (Fig. 2K).

The average prepatent period, determined by faeces examination of twenty-four birds individually, was 110 hr., the range in this group being 108–112 hr.

The oocysts are ellipsoidal and sporulate in 24 hr. at 26° C., the sporulated oocyst possessing a refractile granule (Fig. 2L). Measurements of 150 sporulated oocysts gave a length of $22{\cdot}5 \pm 2{\cdot}3\mu$ and a width of $16{\cdot}25 \pm 1{\cdot}23\mu$, both showing a normal distribution. The length/width ratio was 1·39:1.

### (*b*) *Pathogenicity*

Most workers agree that *E. meleagridis* is non-pathogenic, and my observations confirm this conclusion. Table 1 provides a summary of all the experimental infections carried out with this strain. With one exception, no mortality whatsoever was produced despite the dosing of birds as young as 3 days with massive numbers of oocysts. The single exception was one of a group of eight 6-week-old poults which died on the 5th day after infection with 500,000 sporulated oocysts and in which no obvious cause of death could be found. The experiment was repeated but no death occurred in 10 poults. It is probable, therefore, that death was due to some other cause.

In order to see if infections reduced weight gains, 18 poults were weighed daily from birth and separated into three groups at 2 weeks of age. The birds of each of two groups were then dosed with 25,000 and 100,000 sporulated oocysts, respectively, and the third group were kept as uninfected controls and the weight gains during the course of infection were recorded. The results are presented in Table 2. The infected groups showed slightly lower weights than the controls, but this difference is not statistically significant.

Table 1. *The effect of* Eimeria meleagridis *on poults of various ages*

| Age at dosing (weeks) | Dose (no. of sporulated oocysts) | Mortality* |
|---|---|---|
| 0–1 | 100,000 | 0/6 |
| | 200,000 | 0/4 |
| | 500,000 | 0/4 |
| 2 | 25,000 | 0/6 |
| | 100,000 | 0/6 |
| | 400,000 | 0/10 |
| | 500,000 | 0/8 |
| | 1,000,000 | 0/6 |
| 3 | 200,000 | 0/2 |
| | 1,000,000 | 0/3 |
| 4 | 100,000 | 0/8 |
| 6 | 500,000 | 1/8 |
| | 500,000 | 0/10 |
| | Total | 1/81 |

* Numerator, number of chicks dying; denominator, number of chicks dosed.

Table 2. *The effect of infection on weight gain*

| | Average body wt. (g.) | | | |
|---|---|---|---|---|
| | | Days after dosing | | |
| Group | At dosing | 4 | 8 | 12 |
| Control | 170 | 232 | 313 | 396 |
| 25,000 oocysts | 166 | 214 | 286 | 367 |
| 100,000 oocysts | 164 | 210 | 263 | 350 |

No birds in any experiments showed any symptoms of disease, and all continued eating and drinking quite normally even when infected with very large numbers of oocysts. On the 4th day the normal dark brown caecal droppings were absent from the faeces and did not reappear till the 7th day. Occasionally caseous material was found in the faeces on the 6th day and this was composed of oocysts and epithelial cells. When birds were killed at 84 hr., the caecal contents were paler than usual and on the 5th and 6th day caseous plugs composed of oocysts and much cellular debris were present in the caeca. The caecal contents became normal on the 7th day, but oocysts were still present in large numbers.

Microscopically, eosinophilic cells infiltrated all regions of the intestine early in infection but seemed to be fewer in number than in infections with the other species of coccidia in the turkey (Clarkson, 1958, 1959). By the 5th day the eosinophilic cells were confined almost entirely to the caecal tissues; at this time the parasites had caused a large number of epithelial cells to be discharged into the lumen of the caeca and the glands appeared to have produced a caseous exudate. Despite the destruction of many epithelial cells, however, no haemorrhage was produced and the epithelium rapidly underwent regeneration.

### (c) *Cross immunity*

In the previous papers of this series it has been pointed out how desirable it would have been to confirm the species diagnosis by immunity studies with a known strain obtained from the U.S.A. It was not possible, however, to obtain a strain of *E. adenoeides* or *E. meleagrimitis*, nor could I obtain a strain of *E. meleagridis*. However, since a pure strain of *E. adenoeides* had previously been isolated in this country (Clarkson, 1956), it was possible to carry out immunity tests between this strain and *E. meleagridis* in order to demonstrate that they are two distinct species, although their oocysts are alike. This was of particular importance since in many characters these species are indistinguishable and especially so since a small number of the stages of my strain of *E. meleagridis* were found in the deep glands, which was one of the characters used in the original description of *E. adenoeides* as a new species (Moore & Brown, 1951).

In three separate experiments a total of seventeen birds were rendered immune to *E. meleagridis* by daily dosing with 75,000 sporulated oocysts for 16 successive days, and faeces examinations were carried out by sugar flotation to determine when oocysts were no longer detectable in the faeces. This occurred at from 22 to 26 days after the initial infection. Twelve of these birds were then challenged with *E. adenoeides* (the dose varying from 100,000–500,000 sporulated oocysts) and five were given the same dose of *E. meleagridis*. The latter showed no oocysts in their faeces as a result of infection, but the twelve birds given *E. adenoeides* all passed large numbers of oocysts early on the 6th day and showed oocysts in the lower small intestine, caeca and rectum at autopsy. Sections of these regions confirmed the macroscopic appearance, the ileum being packed with developing stages which I have never seen with *E. meleagridis*. Large numbers of these stages were present in the deep glands as well as in the surface epithelium. This evidence shows that the species described in this paper is immunologically distinct from the pure strain of *E. adenoeides* which had been previously isolated and studied in detail (Clarkson, 1958).

### (d) *Host specificity*

Although Tyzzer (1929) could not infect chickens with *E. meleagridis*, Steward (1947) was able to set up an infection in chickens with turkey coccidia which, on morphological grounds, he believed were *E. meleagridis*. Gill (1954) also claimed to have transmitted *E. meleagridis* to coccidia-free chickens. Hawkins (1952) and Moore (1954), however, believed that *E. meleagridis* is strictly host-specific. I have been unable to infect chickens with large doses of my strain of *E. meleagridis*; a similar result had been previously obtained with *E. adenoeides* (Clarkson, 1956, unpublished data).

## IV. DISCUSSION

Since this strain of *Eimeria* was derived from a single oocyst, only a single species can be present. Oocyst measurements, sporulation time, and general distribution in the intestine suggest that it could be *E. meleagridis*, *E. gallopavonis* or *E. adenoeides*. The other characters of the species described in this paper must, therefore, be compared with previous descriptions of these species in an attempt to make a

specific diagnosis. Most workers agree that *E. meleagridis* is non-pathogenic (Moore, 1954) and my strain did not cause any symptoms of disease and, with one doubtful exception, no deaths resulted from infection. On the other hand, *E. adenoeides* is very pathogenic (Moore & Brown, 1951; Clarkson, 1958). This demonstrates that the strain I have studied is not *E. adenoeides*, and this view is upheld by the absence of cross-immunity and the distribution of the stages in the intestine. Thus the 1st-stage schizonts in the species I have studied develop in the more anterior parts of the small intestine, and the sexual stages in *E. adenoeides* are not only found in the caeca and rectum but also extend for a considerable distance into the lower small intestine (Clarkson, 1958).

This suggests that the strain described here is either *E. meleagridis* or *E. gallopavonis*. Hawkins (1952) was unable to carry out sufficient experiments to determine the pathogenicity of *E. gallopavonis* and thus until this is done this character cannot be used to establish a species diagnosis. The stages in the intestine were described, however, and the main site of development was the rectum, with a few stages in the ileum and caeca. At 4 days large schizonts were present, but Hawkins draws attention to the fact that these schizonts were found in no other site than the rectum. In the strain I have studied the primary site of development at 4 days and later is the caeca, with few stages in the rectum and ileum. The prepatent period of my strain is 110 hr. whereas that of *E. gallopavonis* is 6 days (144 hr.). From the limited evidence available, therefore, it seems unlikely that my species is *E. gallopavonis*.

Tyzzer (1929) stated that development of *E. meleagridis* was rather sharply localized to the caeca, though in young poults stages were found in the small and large intestine. Hawkins (1952) believed that the complete life cycle was passed in the caecum, finding stages there as early as 17 hr. after infection, while at 48 hr. some had divided and mature schizonts were found at $2\frac{1}{2}$–3 days. Hawkins expressed surprise at the small number of stages found in the caeca at 48 hr., but careful examination of other regions did not reveal any stages. Demke (1952) agreed with the caecal site of development though no birds were killed earlier than 70 hr. after infection. Hawkins described the 1st-stage schizont as small and containing only a few merozoites, and the small 2nd-stage schizont, maturing at $3\frac{1}{2}$–4 days, as possessing a residual mass and containing 10–14 merozoites.

I found a large 1st-stage schizont near the rudimentary yolk stalk in the small intestine, maturing as early as 48 hr. and containing many merozoites. The second asexual generation was similar to that of Hawkins, as was the sexual generation, both being found in the caeca, rectum, and a few stages in the small intestine. The finding of the first asexual generation in the small intestine seems an important point of difference from Hawkins's results and its interpretation must be carefully considered. The first possible explanation is that by some means a mixed infection had arisen so that the stages in the small intestine did not in fact belong to the same species as those in the caeca. This explanation was examined very carefully by a number of methods and seems very unlikely for the following reasons:

(i) There has been no evidence of adventitious coccidial infection in coccidia-free controls.

(ii) The same localization of stages in the intestine has been maintained despite many oocyst passages and the selection of caecal oocysts alone for infecting other poults.

(iii) If the 1st-stage schizonts were another species one would expect more second generation and sexual stages in the yolk-stalk region, whereas I have never seen a single stage in histological sections other than the first generation, and this can readily be distinguished by the large 'globule'.

Table 3. *Characters of* Eimeria adenoeides, E. gallopavonis *and* E. meleagridis (*British and American*)

| Characters | | | *E. adenoeides* | *E. gallopavonis* | *E. meleagridis* American | *E. meleagridis* British |
|---|---|---|---|---|---|---|
| Oocyst | Size ($\mu$) | Length | 21·50–30·00 | 22·2–32·7 | 20·3–30·8 | 18·20–28·20 |
| | | Width | 13·50–19·50 | 15·2–19·4 | 15·4–20·6 | 13·00–20·70 |
| | | Mean | 25·68 × 16·25 | 27·1 × 17·2 | 24·4 × 18·1 | 22·50 × 16·25 |
| | Shape | | Ellipsoidal | Ellipsoidal | Ellipsoidal | Ellipsoidal |
| | Refractile granule | | Present | Present | Present | Present |
| | Sporulation time (hr.) | | 24 | 24 | 24 | 24 |
| Localization in intestine | | | Lower ileum, caeca and rectum: deep glands as well as villi | Rectum, ileum and caeca: tips of villi | Caeca, ileum and rectum: tips of villi | 1st stage in small intestine, remainder caeca: deep glands as well as villi |
| Morphology of stages and site in epithelial cell | Asexual | 1st: large, below cell nucleus | | Small, above cell nucleus | Small, above cell nucleus | Large, below cell nucleus |
| | | 2nd: small, above cell nucleus | | (*a*) Small, above cell nucleus<br>(*b*) Large, below cell nucleus, in rectum only | Small, above cell nucleus | Small, above cell nucleus |
| | Sexual | Above cell nucleus | | Above cell nucleus | Above and below cell nucleus | Above cell nucleus |
| Prepatent period (hr.) | | | 114–132 | 144 | 120 | 110 |
| Pathogenicity | | | Very pathogenic | Undetermined | Non-pathogenic | Non-pathogenic |

(iv) Despite a very careful search no stage has been seen in the caecum before 52 hr. and even then there were only small trophozoites.

(v) The stages seen in the small intestine do not fit in with any other described species.

(vi) On two occasions I took the very small number of oocysts obtained by scraping the mucosa of the small intestine in the yolk-stalk region, and fed them to coccidia-free birds. A typical caecal infection developed by the 5th day.

From this evidence (Table 3) it seems reasonable to conclude that the strain in question is *E. meleagridis* and that the 1st-stage schizont of this species does

occur in the small intestine despite the findings of Hawkins (1952), whereas the rest of the life cycle is passed in the caecum. This is not unique, as both *E. necatrix* in the chicken (Tyzzer, Theiler & Jones, 1932) and *E. bovis* in the ox (Hammond, Bowman, Davis & Simms, 1946) have a similar life cycle. It would seem possible that the stages which Hawkins found in the caecum at 17 hr. were another species, since he did not use a strain derived from a single oocyst. His failure to find stages elsewhere in the intestine is not unusual, as large doses of oocysts must be given to detect the 1st-stage schizont, and in a few birds I have not managed to find any stages in histological sections made early in infection. A small number of second asexual and sexual stages were found in the caecal glands and so this character alone will not suffice to differentiate from *E. adenoeides*.

Even though *E. meleagridis* is not pathogenic it produces very marked lesions in the caeca in the form of white caseous plugs, composed largely of oocysts and dead epithelial cells. This is of some importance in the diagnosis of turkey coccidiosis in the field as the lesions in *E. adenoeides* infection are somewhat similar but extend some distance into the lower small intestine (Clarkson & Gentles, 1958).

The isolation and study of *E. meleagridis* from turkeys in Great Britain, together with previous observations on *E. adenoeides* and *E. meleagrimitis* (Clarkson, 1958, 1959), have provided some indication of their differential features and relative pathogenicity.

## V. SUMMARY

A description is given of the life history and pathogenicity of a species of *Eimeria*, derived from a single oocyst infection in a coccidia-free bird, and subsequently identified as *E. meleagridis*.

There are two asexual generations followed by the sexual generation. The first schizogony cycle occurs in the small intestine and the second and sexual generations are restricted to the caeca.

The organism is not pathogenic but the sexual stages produce marked caseous lesions in the caeca. It is immunologically distinct from a pure strain of *E. adenoeides* and cannot be transmitted to chickens.

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# SEASONAL VARIATIONS IN THE INCIDENCE AND DEVELOPMENT OF THE CESTODE *PROTEOCEPHALUS FILICOLLIS* (RUD. 1810) IN *GASTEROSTEUS ACULEATUS* (L. 1766)

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(With 3 Figures in the Text)

The life-cycle of *Proteocephalus filicollis* (Rud. 1810) was described by Meggitt (1914), who showed experimentally that the procercoid developed in the haemocoele of *Cyclops varius*, infection of which probably occurred by the ingestion of embryonated eggs. The cycle was completed when an infected copepod was eaten by a stickleback and the procercoid grew into the adult tapeworm. Subsequently, the life-cycles of several other species of *Proteocephalus* have been described (Neiland, 1952; Wagner, 1953; and review of earlier work by Wardle & McLeod, 1952).

The weakest link in the experimental evidence concerning the life-cycle appears to be the fate of the procercoid when an infected *Cyclops* is eaten by a fish. Wardle & McLeod (1952, p. 184) write '...the procercoids invade such organs as the liver, gut, muscles, etc., developing there into typical plerocercoids with invaginated holdfasts'. The evidence for this statement is discussed later, but in the species recently investigated no such extra-intestinal phase has been reported.

The investigations described in this paper were carried out to determine (*a*) the position of *Proteocephalus filicollis* in the fish host throughout development from a recently ingested procercoid to a gravid worm; (*b*) the rate of growth; and (*c*) the correlation, if any, between size of worm, period of year and stage of development reached. Such a correlation between time of year and stage of maturity reached has been shown to exist in several cestodes parasitic in freshwater fish of the temperate zone, e.g. *Corallobothrium giganteum* and *C. fimbriatum* in *Ictalurus punctatus* (Essex, 1927); *Triaenophorus crassus* in *Esox lucius* (Ekbaum, 1937); *T. crassus* and *T. nodulosus* in *E. lucius* (Miller, 1943); *Proteocephalus stizostethi* in *Stizostedion vitreum vitreum* (Connor, 1953).

In the course of the survey, which extended over two years and involved the examination of 1620 sticklebacks, a considerable amount of information about the monthly variations in incidence and intensity of infection of *Proteocephalus filicollis* was obtained. From a consideration of these data certain hypotheses are advanced concerning the factors, both intrinsic and extrinsic, which affect the growth, development and incidence of this tapeworm in *Gasterosteus aculeatus*.

## MATERIAL AND METHODS

The tapeworm was found occurring as a natural infection of the three-spined stickleback (*Gasterosteus aculeatus*) living in a lochan of approximately 1000 m.$^2$, situated near Bellshill, North Lanarkshire. The lochan is completely isolated from other water, and no streams enter or leave it. It is comparatively shallow, not exceeding 1·5 m. in depth, and extremely eutrophic. The only fish other than *G. aculeatus* found in the lochan was 1 *Perca fluviatilis*.

The incidence of the tapeworm was estimated during the 2-year period August 1956 to September 1958 by the monthly examination of about 60 fish. The fish were caught by hauling a light trawl (4 ft. beam, 2 ft. deep, 5 ft. long) across the lochan. In winter a 'lead' was first opened in the ice. No difficulties were experienced in obtaining fish until an epidemic of *Ichthyophthirius* sp. destroyed most of the fish in September 1958. During the coldest months, the trawl was soaked well and hauled slowly to permit the weighted heel rope to dig into the surface layers of the bottom mud where most fish were located.

The fish were usually kept for 18 hr. in running-water tanks, during which time much faecal matter was discharged, and this simplified the subsequent examination of the intestine. Fish were killed by pithing and the abdomen opened ventrally with a cut extending back to the anus. The intestine, from stomach to rectum inclusive, was removed with forceps, placed in a drop of saline on a 3 in. × 2 in. slide and examined under light pressure from a covering 2 in. × 1 in. slide, using transmitted light and × 16 magnification.

Permanent preparations of worms were made by fixing in a corrosive sublimate and staining with Gower's carmine; or by fixing in 70% alcohol and staining with 0·1% catechol (Johri & Smyth, 1956).

## RESULTS

Details of the number of fish infected with *Proteocephalus filicollis*, and the size, stage of maturity, and position in the intestine, of the worms throughout the year are shown in Table 1.

### (i) *Growth and development*

The size of the worms (Table 1, col. 4) is only an approximation, made by measuring the worms alive under gentle pressure (see Methods). It is of significance chiefly in indicating: (*a*) that very small (young) worms are still to be found from July to November, (*b*) few worms exceed 5–6 mm. in length (the size at which strobilization first occurs) until the following spring, and (*c*) that growth is very rapid from April onwards.

Except in the months of August and September, when all the worms found were small immature unsegmented plerocercoid stages, there was a considerable scatter in the degree to which development had proceeded (column 6). This was correlated with size; thus worms started to show segmentation when they were 5–6 mm. long and genital rudiments became distinct between 6 and 8 mm. The number of worms to have reached these levels of development is indicated in column 6 as S, i.e. the

Table 1

| Date of sample (1) | No. of fish (2) | No. infected (3) | Mean size of worm (mm.) (range) (4) | Position in gut (5) | Condition and no. of worms (6) |
|---|---|---|---|---|---|
| 30. xi. 56 | 50 | 25 | 1·5 (0·5–6·0) | — | Immature; 75<br>2 S: 1 GR |
| 15. i. 57 | 53 | 15 | 2·9 (1·0–8·0) | Rectum | Immature; 25<br>4 S: 3 GR |
| 28. ii. 57 | 61 | 13 | 2·4 (1·5–7·0) | — | Immature; 28<br>3 S: 2 GR |
| 29. iii. 57 | 80 | 10 | 3·0 (1·5–7·5) | — | Immature; 9<br>Mature; 4 |
| 22. iv. 57 | 62 | 6 | 5·0 (1·0–8·0) | — | Immature; 4<br>Mature; 3 |
| 7. vi. 57 | 67 | 1 | 18·0 | Small int. | Gravid; 1 |
| 2. vii. 57 | 52 | 3 | 20·0 | Small int. | Gravid; 3 |
| | | 5 | 0·4 | Rectum | Immature; 8 |
| 6. viii. 57 | 50 | 22 | 1·0 (0·3–2·0) | Rectum | Immature; 32<br>0 S |
| 19. ix. 57 | 80 | 30 | 1·9 (0·5–3·5) | Rectum | Immature; 60<br>1 S: 0 GR |
| 16. x. 57 | 60 | 25 | 2·5 (0·5–5·0) | Rectum | Immature; 31<br>2 S: 2 GR |
| 14. xi. 57 | 62 | 24 | 2·7 (1·0–5·0) | Rectum | Immature; 32<br>2 S: 1 GR |
| 17. xii. 57 | 60 | 9 | 3·3 (1·5–6·0) | Rectum/ Small int. | Immature; 18<br>4 S: 1 GR |
| 17. i. 58 | 60 | 19 | 2·7 (1·5–10) | Rectum/ Small int. | Immature; 26<br>4 S: 1 GR |
| 18. ii. 58 | 63 | 6 | 3·5 (2·5–6·0) | Rectum/ Small int. | Immature; 8<br>2 S: 0 GR |
| 14. iii. 58 | 61 | 8 | 2·2 (1·0–4·0) | Rectum | Immature; 11<br>1 S: O GR |
| 22. iv. 58 | 66 | 1 | 5·0 (4·0–6·0) | Small int. | Immature; 2<br>2 S: 2 GR |
| 7. v. 58 | 60 | 2 | 12·0 (10–14) | Small int. | Mature; 2 |
| 3. vi. 58 | 68 | 3 | 20 (14–30) | Small int. | Mature; 3<br>Gravid; 3 |
| 25. vi. 58 | 60 | 1 | 28 (26–30) | Small int. | Gravid; 2 |
| 29. vii. 58 | 62 | 12 | 0·7 (0·3–1·3) | Rectum | Immature; 18<br>0 S |
| 21. viii. 58 | 139 | 58 | 0·9 (0·3–2·5) | Rectum | Immature; 169<br>0 S |
| 29. ix. 58 | 74 | 34 | 1·0 (0·3–3·0) | Rectum | Immature; 66<br>0 S |

The incidence, size, location, and stage of development reached by *Proteocephalus filicollis* in the 3-spined stickleback, determined at monthly intervals from November 1956 to September 1958. Column 5 indicates the region where most scolices were attached. Column 6 shows the stage of development, total number of worms, and the number of immature worms showing segmentation (S) and genital rudiments (GR). Int., intestine.

number of worms recovered showing segmentation, and GR, i.e. number of worms showing genital rudiments. The latter is rather an arbitrary division, and in fact meant the presence of a darkly staining mass of cells in the mid-line of the proglottid. These appeared shortly after segmentation, but it is possible that other genital rudiments, not so clearly visible in whole mounts, preceded it and may be present before segmentation commences. Worms over 8 mm. long showed both male and female genitalia and are described as mature. Only a few gravid worms have been found and these all exceeded 10 mm. and were usually 1·5–3·0 cm. in length. It can be seen from the data that the development of *P. filicollis* occurs as an annual cycle, with a few gravid 1-year-old worms just overlapping the new season's plerocercoids.

(ii) *Position in host*

The regions in which the scolices of the worms were attached is shown in Table 1 (column 5). Where only one region is recorded, 80 % or more of the worms occurred in that region. In several cases worms attached in the small intestine had strobilae which extended into the rectum or even hung from the anus (cf. Miller, 1943). Again, as with development, there appeared to be a close correlation of position with size. Small immature worms (August–December) are found in the rectum, larger mature and gravid worms are attached in the small intestine, almost up to the pyloric valve.

(iii) *Incidence*

The monthly incidence of *P. filicollis* in the fish population from November 1956 to September 1958 (calculated from cols. 2 and 3, Table 1) has been plotted as a percentage on a logarithmic scale in Fig. 1. A logarithmic scale was used as the data refer to population changes and it is apparent that a loss of (say) 10 worms from a population of 20 is very different from a loss of 10 worms from a population of 200. The plot drawn through the points is somewhat arbitrary, particularly during the early summer months when the incidence is low and a variation of even 1 from the 'expected number' of fish infected causes, on a log. graph, a very large apparent difference between 'expected' and 'measured'. The number of infected fish found in the July 1957 sample is divided into two categories (Table 1). The first is the number infected with 1-year-old tapeworms (gravid, and with scolices attached close to the pyloric valve), the second the number of fish infected with recently acquired plerocercoids. These are plotted separately in Fig. 1, using the appropriate year class symbol.

The results show a strongly marked seasonal cycle in the incidence during 1956–7 which was closely reproduced by the 1957–8 year class, and to judge from the 3 months of survey completed before the fish population crashed (§ vi*d*), the cycle was being repeated by the 1958–9 year group of worms.

(iv) *Incidence and size of host*

The monthly distribution of *P. filicollis* infections according to the size of the host fish is shown in Figs. 2 and 3. The October and November 1957 histograms show that it is the 2–3 cm. group ($0^+$ fish) that are the most heavily infected, a result which the July and August 1958 figures confirm. The difference in the

parasitization rate between $0^+$ and $1^+$ fish is, however, only one of degree; in this respect the December result is obviously abnormal

The higher incidence in the smaller fish becomes less distinct from January onwards but this is because the $0^+$ fish now constitute the majority of fish in all except the largest categories, and the year classes can now no longer be separated easily by length. This is best shown by the March–June 1958 histograms in which all the fish caught in a haul were measured, and not as previously just those dissected.

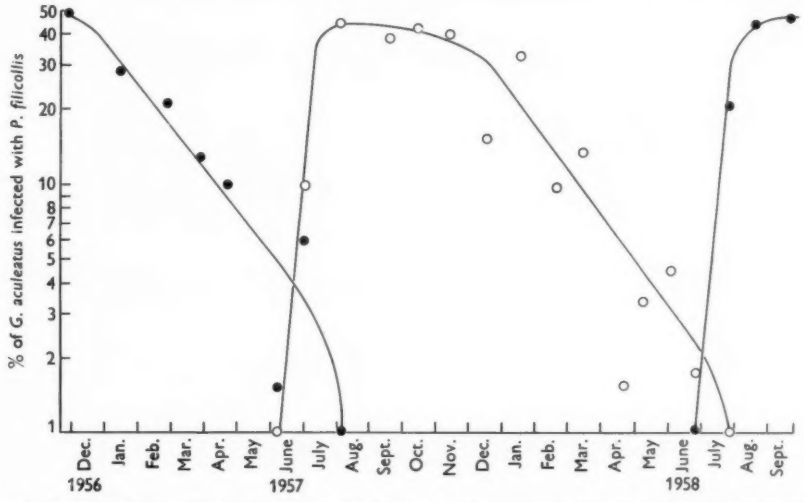


Fig. 1. The incidence of *Proteocephalus filicollis* in the three-spined stickleback *Gasterosteus aculeatus*) between November 1956 and September 1958. ●, incidence in the 1956–7 and 1958–9 year class; ○, incidence in the 1957–8 year class.

The February–June histograms show the fall in incidence which occurs during this period and indicates that the chance of a worm surviving to maturity is independent of the size of the fish in which it occurs. The cataclysmic destruction of the 1958 fish class (September, Fig. 3) is reported further in § vi.

### (v) *Intensity of infection*

The numbers of worms found in infected fish were recorded, and the average worm burden, i.e. the total number of worms divided by the number of infected fish, was calculated for each month. The results showed little seasonal variation other than a peak in September 1957 of 2·1 and in August 1958 of 2·9, and a trough in May and June. During the rest of the year both in 1956–7 and 1957–8 the intensity index usually fluctuated around 1·3–1·6.

Infections of the same number of worms were grouped together for the months August, September, October and November. These months were chosen as the incidence remains fairly steady, between 40 and 50 % (Fig. 1), during this period. The results were, out of 448 fish, 265 were uninfected, 113 had single worm infections, 37 were infected with 2 worms, 18 with 3, 10 with 4, 5 with 5 and 7 with between 6 and 12. The Poisson series, assuming random feeding by

sticklebacks on infected copepods each carrying only a single procercoid, predicts a distribution of 226 uninfected, 155 with 1 worm, 53 with 2, 12 with 3 and 2 with 4 or more. The implications of the discrepancies between these two series are discussed later.

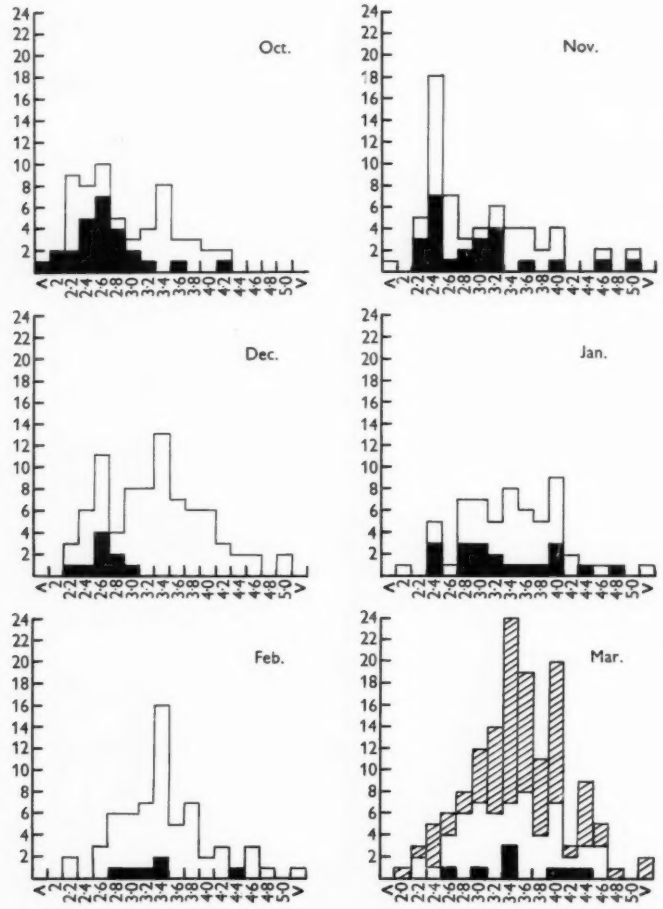


Fig. 2. The distribution of worms in the fish population according to size of the fish from October 1957 to March 1958. Black area shows number of infected fish; white the number of fish examined; shaded the size distribution of all fish caught (only recorded from March onwards). The ordinate shows number of fish, the abscissa the length of fish in centimetres. Each group is 2 mm. except the first column which is all fish less than 2·0 cm. and the last which is all fish over 5·1 cm.

### (vi) *Other observations relating to the biology of* Proteocephalus filicollis

(*a*) *Intermediate host.* Copepods recovered from the intestine of fish caught in September 1958 were examined by Dr Bruce who reported *Cyclops vernalis* and *C. albidus* as the most abundant species; in plankton hauls *C. agilis*, *C. macruroides*

and *Diaptomus gracilis* were also found in large numbers. It is probable that one or more of these species is involved in the transmission of *P. filicollis*.

(*b*) *Abnormal development.* Of the 670 worms examined all except one appeared morphologically normal. The exception was a plerocercoid, 2 mm. in length, which had a normal scolex and unsegmented body for the first 1·6 mm., but posterior to this the worm was completely divided, by a median cleavage, to form two 'tails', each 0·37 mm. long.

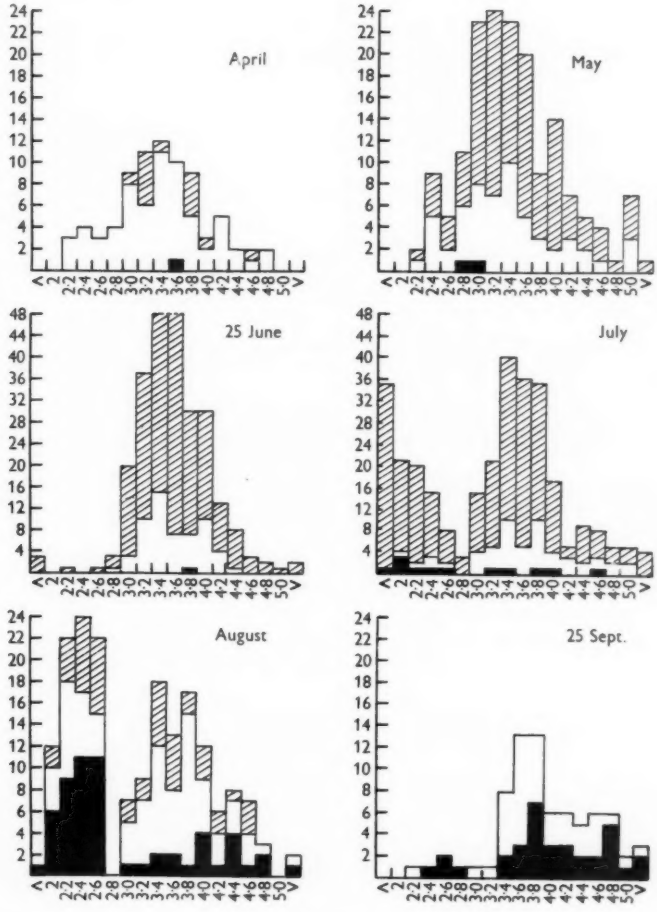


Fig. 3. The distribution of worms in the fish population according to size of the fish from April to September 1958. For explanation see Fig. 2. Note scale on the ordinate is halved for June and July.

(*c*) *Structure of the egg-shell.* Mature and gravid worms were fixed in 70% alcohol for 1 day, washed and placed in 0·1% catechol for 1 hr. at 40° C. (Johri & Smyth, 1956). Both the eggs and vitellaria gave a strong reaction indicating the

presence of polyphenol oxidase, and therefore, almost certainly, the same mechanism of egg-shell formation as occurs in pseudophyllidean cestodes, and in monogenic and digenic trematodes (Smyth, 1956; Johri & Smyth, 1956).

(*d*) *Intercurrent infections.* No other helminth was found in the intestine or body cavity of the 1620 fish examined, but one larval nematode was found partially encapsulated in the liver.

All fish in which the eyes were examined (approximately 250) were infected with *Diplostomum spathaceum* metacercariae in the lens, and most of them had lighter infections of metacercariae of another species of *Diplostomum* in the retina.

Ectoparasites included *Gyrodactylus* sp. (incidence in late summer months over 50 %), and a ciliate, probably *Cyclochaeta* sp. (incidence in summer months 100 %).

During August 1958 the ciliate *Ichthyophthirius* (sp. *multifillis?*) became established and increased at a great rate. By 5 September the $0^+$ population had been reduced to 25 % of the total compared with over 60 % in 1957, and by 25 September (Fig. 3) the 1958 year class had nearly been destroyed. Considerable mortality also occurred in $1^+$ and $2^+$ fish. Up to this time only one haul had been necessary, except occasionally in mid-winter months, to collect between 80 and 500 fish. In early September repeated hauls had to be made and averaged only 18 fish per haul, by late September the average had dropped to 7, by 25 October to 2, and by 15 November to 1·5.

(*e*) *Maturation of worms in immature host.* This could not be investigated in *P. filicollis* as the sticklebacks mature as soon as the worms, but 1-year-old fry of *Coregonus clupeoides* were kindly provided by Mr Hamilton of this Department. Most of these fish, which had been fed on plankton, were infected with a species of *Proteocephalus*. Many plerocercoid stages were found in the intestine; some mature and two gravid worms were also recovered. There can be no doubt that in this species the worm can mature in an immature host.

## DISCUSSION

### (*a*) *Attachment and migration in the fish host*

In the summer and autumn, procercoids of *P. filicollis*, about 0·3 mm. in length, ingested in infected copepods, are released into the lumen of the intestine. No penetration of the intestine wall occurs. The plerocercoid, as it may now be called, attaches itself to the gut wall, usually in the rectum, by means of its suckers. The reason for the preference for the rectum as a site of attachment is unknown, but seems, at first, a peculiar modification for an animal dependent on the products of host digestion. The rectum of a fish, however, is very different physiologically from the rectum of a land animal (Barrington, 1957; Al-Hussaini, 1949), and it is probably a fairer comparison to equate this region with the posterior part of the ileum of a mammal. Although no rigid correlation between size and position in the gut existed, over 80 % of the worms less than 5 mm. in length were found in the rectum. Worms over 5 mm., which show segmentation, had their scolices attached in the posterior part of the small intestine, and the scolices of gravid

worms lay close to the pyloric valve. The same phenomenon of initial attachment in a posterior region, followed by a forward migration prior to maturation, has been recorded in *Diphyllobothrium* developing in rats (Archer & Hopkins, 1958).

The fact that plerocercoids of *P. filicollis* occur in the rectum as a normal part of their life-cycle, casts some doubt on the validity of the conclusions reached by Hunter (1929) on the life-cycle of *P. pinguis*. Hunter found a proteocephalan plerocercoid in the 'lower part of the digestive tract of the yellow perch', and concluded that occurrence in such a situation indicated the worm was in an abnormal host. At the same time he found *P. pinguis* in three pike 27–41 cm. long, which he considered would not be eating entomostraca. With these two observations in mind, Hunter attempted to demonstrate that there was an intermediate fish host in the life-cycle of *P. pinguis*. In retrospect, it seems doubtful if his experiments were conclusive, and the whole question of intermediate hosts in the proteocephalan life-cycle needs re-investigation. The types of life-cycles can be divided into three categories:

(1) *Direct cycle only*. Procercoids develop through the plerocercoid stage to the adults in the intestine of the definitive host.

(2) *Indirect cycle* (*facultative*). In this category infection of the definitive host, a carnivorous fish, may occur directly or: (i) By cannibalism (this may become the predominant source of infection in older fish). (ii) By eating fish of a different species in which the plerocercoid is established in the intestine.

(3) *Indirect cycle* (*obligatory*): (i) Plerocercoids occur in the tissues of the definitive host, returning to the intestine when a certain stage of development is reached (active return). (ii) Plerocercoids in the tissues of the definitive host species, returning only when the tissues are eaten in the course of cannibalism (passive return). (iii) Plerocercoids in the tissues of a species of fish other than that of the definitive host.

The term intermediate host is best restricted to categories 3(ii) and (iii); in the other cases the term transport or accommodation host is preferable, as it implies more accurately the relationship.

To review the evidence for the existence of these different life-cycles is not possible here, as many of the conclusions presented in earlier papers necessarily need revision in the light of later work, but one paper (Hunter, 1928) on the life-cycle of *P. ambloplitis* is of particular interest because, in describing the life-cycle via a tissue phase, it raises, incidentally, a very fundamental issue. Hunter describes the procercoid stage of *P. ambloplitis* as boring through the gut wall and developing in the liver, mesenteries and gonads. This is interesting, but what is extraordinary is that this occurred when fish were added to the aquarium 3 days after the eggs, i.e. at that time the procercoids were at the most 3 days old. The fish were left 8 days, but even by the 11th day the procercoids, if they develop at a rate comparable with other species, would not be infective. The details given by Hunter of the size of the procercoid and lack of a cercoma (Hunter, 1929), when compared with Wagner's (1954) account of development in the copepod host, indicate that no fully developed procercoid existed. The question arises,

can procercoid development, cut short by the copepod being eaten, continue, at least for some time, in the tissues of a fish? *A priori* it seems improbable, but Hunter's results are difficult to interpret otherwise. It would, of course, be of interest to repeat his experiments and let procercoids develop fully before feeding them to fish and observe whether the procercoid still penetrates the gut wall.

### (*b*) *Development and maturation*

In *P. filicollis*, as in many other tapeworms parasitic in freshwater fish of temperate climates, maturation shows a seasonal cycle. The time of appearance of proglottids and genital rudiments is nearly simultaneous (Table 1); in fact, with the methods used it is not possible to determine precisely when either process starts. As Haderlie (1953) recorded, the plerocercoid goes through a rugose phase which is difficult to differentiate in the living worm from early proglottids. Even in stained whole mount preparations the banded effect brought about by the clustering of nuclei merges imperceptibly with the region where distinct proglottids exist. Meanwhile, in each 'block of nuclei' genital rudiments appear. No correlation between these two processes has ever been demonstrated, but clearly an understanding of the mechanism, whereby a 4 mm. long, unsegmented plerocercoid grows into a 6 mm. long, segmented worm with genital primordia, is of fundamental importance. The time of year at which proglottids and genital primordia appear varies greatly in different worms, but there can be little doubt that this is due to the temperature falling in September and so checking growth and development at the plerocercoid stage until the following spring. A few of the plerocercoids, presumably those picked up in July, however, do reach the stage of early strobilization and genitalia before the near zero (°C.) temperatures of January–March check all growth (Table 1, and Wagner, 1954). The completion of development in April, May and June fits in with this simple temperature control hypothesis.

The interrelationship of maturation and temperature, however, merits a thorough investigation. For instance, does a lowering of the temperature merely slow development, or is there a relatively high threshold level, below which somatic growth may continue but no genital development occurs? It would be of particular interest to compare this information with that from *Schistocephalus* and *Ligula*. In both these pseudophyllideans, temperature is used as the sole trigger mechanism to initiate spermato- and oogenesis. Maturation is prevented in the fish host as the temperature never reaches 33° C. (Smyth, 1952). Has this physiological mechanism, to prevent maturation occurring until the warm-blooded definitive host is reached, evolved by the simple process of modifying (by raising the threshold temperature) a control mechanism already widespread in more primitive cestodes?

### (*c*) *Incidence*

Although the existence of an annual developmental cycle in fish tapeworms has been recorded many times, an annual cycle in the incidence level has only been recorded as the result of gravid worms being shed at a particular time of year, and the fish building up new infections shortly after, or in some cases several

months later. The significant point in the host/parasite relationship, which has been overlooked, is that the incidence throughout the year is a dynamic equilibrium between loss and gain. This is clearly shown in Fig. 1, in which it can be seen the incidence does not just rise while fish are eating infected copepods and then remain static until the worms become gravid and are lost, but shows considerable changes long before the worms are mature. In fact, in *P. filicollis* the loss of 'spent' worms accounts for the death of an insignificant fraction of the total. Less than 1 % of the worms to become established survive to become gravid (as shown by comparing the product of incidence × intensity in August with that of May—when gravid worms first appear) and, if certain calculations given here are accepted, the survival figure is probably lower still, around 0·5 %. In other words, of every 200 worms to become attached in the intestine, only one reaches full maturity; the 199 die as a result of unknown causes.

The simplest interpretation of the incidence cycle shown in Fig. 1 is that a rapid rise occurs in July, due to the picking up of new infections, which reaches a peak in August or September. This is followed by a period of 4 months when conditions are favourable for the worms and few are lost. During the winter months increasingly adverse conditions, such as shortage of food and low temperatures, cause a high mortality amongst the worms. (This is not to be confused with the effect these conditions may have on the host, which by affecting one age group of fish more than another, may affect the overall incidence.) Finally, in late spring the return of more favourable conditions leads to the establishment of a new, and of course much lower, incidence level, which remains constant until the worms become gravid and leave the fish.

This interpretation, however, only fits the observations in a superficial manner. It assumes that the incidence is constant between August and November because no loss or gain occurs, whereas it could be due to loss equalling gain. Indeed, the presence of young plerocercoids in the fish up to November (Table 1) is evidence that new infections are occurring, and hence, since the incidence is constant, loss of worms must also occur. The difference between the host-parasite relationship in autumn and in winter is therefore not a qualitative one. In winter the intestine does not suddenly become an unfavourable environment; at the most the change is of a quantitative nature, which leads to a process already occurring being speeded up. Unfortunately, without detailed information on the speed of growth of plerocercoids at different temperatures, it is impossible to assess accurately the number of worms in the autumn samples which were less than 1 month old, and hence to be able to calculate directly the monthly gain and loss. After November, however, the rate of loss is known (Fig. 1), as no new infections occur until the following July. The main point at issue during this latter period is the rate at which worms are lost. This is an extremely important point to establish accurately if deductions about the physiological cause of worm loss are to be made.

It can be seen (Fig. 1) that the incidence figures for December to May 1956–57 and 1957–58 fit quite closely a straight line, which on a logarithmic scale implies a constant rate of loss. But, if this is a correct interpretation, it means the

hypothesis that the loss of worms was due to increasingly adverse conditions during the winter is no longer probable. A constant rate of loss throughout the period December to May makes it unlikely that the cause of worm loss is a seasonably dependent factor. From this point, it seems reasonable to extrapolate one step further and suggest that the loss of worms, which it has been shown must occur during August to November, also occurs at approximately the same rate.

Although this last step is unsubstantiated, its acceptance makes possible a very simple explanation of the observed variations in incidence. The reason the incidence level ceases to rise after August is not because infected copepods have disappeared, but because by that time a sufficiently high concentration of worms in the fish is reached for the rate of loss to equal the rate of uptake. By December, the full effect of the physiological factors which cause loss of immature worms starts to show, as it is no longer masked by new infections, and approximately 30 % of the worms are lost every month thereafter. This leads to very big drops in the incidence level at first (during the winter months) but the arithmetic differences in incidence levels are small by March to June which gives the illusionary impression that the rate of loss is slowing in spring.

(*d*) *Intensity of infection and its bearing on the copepod parasite relationship*

The distribution of worms in fish according to the intensity of infection is shown in § 4 of the results, together with a Poisson series. The latter assumes random feeding by sticklebacks on infected copepods and that infected copepods carried only a single procercoid. Neither of these assumptions was likely to be strictly true but the comparison was made to determine how widely the two series diverged. The fit between the two series, as can be seen, is poor, but a slight modification gives a much better fit.

The distribution of infections according to the size of the fish during the months July to December, when $0^+$ and $1^+$ fish are easily separable (Figs. 2, 3), shows that the incidence of *P. filicollis* in $1^+$ fish is much lower than in $0^+$ fish. This difference in incidence probably arises because of different feeding habits. This conclusion is supported by the observation that during late summer and autumn $0^+$ fish have mainly zooplankton in their intestines, particularly *Chydorus sphaericus* and *Cyclocypris ovum*, whereas $1^+$ fish are feeding more commonly on algae and chironomid larvae.

No evidence of immunity developing as the result of a present or past infection was observed, in fact many of the multiple infections were of worms of greatly different ages. Similar observations have been recorded by most other workers, only one of whom (Wagner, 1954) suggested that a challenge infection led to the loss of both established worms and challenge-dose worms.

If, then, it is assumed that many of the $1^+$ fish eat so few copepods that their chances of consuming an infected one are very small, these fish should be deducted from the total and the distribution of worms calculated in the remainder. A Poisson distribution of the worms, assuming 30 % of the fish are not competing for copepods, is 266 uninfected, 110 with 1, 52 with 2, 19 with 3, 5 with 4. The observed distribution was respectively 265, 113, 37, 18 and 10. The fact that the number of

uninfected fish is the same has no significance as this was arrived at by assuming 30% of the fish rarely fed on copepods. What is of significance is that, having tied together the predicted and observed number of uninfected fish, the rest of the observed series shows a close fit to a Poisson distribution. The only discrepancy is that a small number of heavy infections with five or more worms occurred. It is probable, therefore, that the second hypothesis, that most infected copepods carry only a single procercoid, is true, otherwise a much larger number of multiple infections would be expected. This result is not surprising, as a distribution of parasites in a population showing, as *Proteocephalus* does, a relatively high incidence but low infectivity is likely to arise only if the source of infection is essentially single infective forms. Essex (1927), while working on species of the closely allied genus *Corallobothrium*, found as a result of direct examination that no copepod infected in nature carried more than one procercoid. However, this is not to imply that multiple infections cannot occur; most, if not all workers, who have experimentally infected copepods record multiple infections, some with up to 50 procercoids. The significance of this low intensity of infection in *Cyclops* in nature is the implication that the odds against a *Cyclops* becoming infected is very great indeed. Such would hardly be the case if Hunter's (1928) suggestion that the movements of the onchosphere within the egg-shell attracted *Cyclops*. Indeed it is extremely improbable that the acuity of the *Cyclops*' eye is sufficient to observe such movement. It seems more probable that Essex's (1927) description of *Cyclops* grazing over the surface, eating the outer gelatinous shell and rejecting the egg, except for the occasional accidental ingestion, comes nearer to a true picture of the mode of infection. If this is the case the enormous advantage of the free-swimming coracidia of the pseudophyllian worms becomes the more apparent.

## SUMMARY

1. The incidence, intensity of infection, rate of growth and genital development in *Proteocephalus filicollis* has been studied by the monthly examination of approximately 60 *Gasterosteus aculeatus* over a period of 2 years.

2. The existence of an annual cycle, commonly reported for cestode parasites of fish in a temperate climate, is clearly established. The most advanced stages commonly occurring are: plerocercoids (0·25–5 mm.) July–November; strobilate worms with genital primordia (5–8 mm.) December–April; mature worms (1 cm.) April–May; gravid (2 cm.) June–July.

3. No tissue phase occurs between the time the procercoid is swallowed and the mature worm develops, but there is a migration from the rectum to the anterior part of the intestine.

4. From a consideration of the seasonal variation in incidence it is deduced that the parasitic population is in dynamic balance and that approximately 1% of worms present are lost daily.

5. No evidence to support the existence of immunity was found. The lower incidence in $1^+$ than in $0^+$ fish was attributed to feeding habits.

6. Other aspects discussed are the interrelationship of proglottid formation and appearance of genital rudiments; the structure of the egg-shell; and the

intensity of infection of *Cyclops* with procercoids as calculated from the numerical distribution of worms in the fish host.

7. Reference is made to intercurrent helminth and protozoan infections and the effect of an epidemic of *Ichthyophthirius* sp. on the fish population.

I wish to thank Prof. C. M. Yonge for granting every facility for this work. My thanks are also due to Mr Archer, Miss Bell, Mr Berrie, Miss Spence and Miss Wyllie for help in collecting fish, and to Mr Gray who showed me the lochan.

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# NUCLEAR DIVISION IN THE TROPHIC STAGES OF *IODAMOEBA BÜTSCHLII* (PROWAZEK, 1912) DOBELL, 1919

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(With Plates VIII–X)

## INTRODUCTION

The first description of *Iodamoeba bütschlii* was made by von Prowazek (1912), who provisionally designated the organism *Entamoeba bütschlii*. On the basis of nuclear morphology, as well as the conspicuous iodine vacuole in the cyst, Dobell (1919) later established a new genus, *Iodamoeba*, and renamed the amoeba *I. bütschlii*. Among the investigators of *I. bütschlii* Dobell (1919), Taliaferro & Becker (1922), and Wenrich (1937) made extensive morphological studies and presented detailed drawings depicting nuclear structure. The process of nuclear division, however, has not been elucidated, due in part to difficulties encountered with cultivation *in vitro*, and with attempts to infect laboratory animals, as well as reflecting the usual scarcity of trophic stages in voided stools. Von Prowazek (1912) labelled nuclear division in this amoeba as 'promitotic', but his description is too brief for evaluation. Wenrich (1937) presented a figure (fig. 67) which was believed to represent one stage of division of *I. bütschlii* and predicted that the chromosomes were formed from the periendosomal chromatin granules, inasmuch as the granules were known to stain positively by the Feulgen technique, as Reichenow (1928) first demonstrated.

Of the six species of amoebae living in the digestive tract of man, the process of nuclear division has been described for all except *I. bütschlii* (Dobell, 1919, 1928, 1938, 1940, 1943; Kofoid & Swezy, 1925; Child, 1926; Uribe, 1926; Cleveland & Sanders, 1930; Wenrich, 1939, 1944; Stabler, 1940; Pan & Geiman, 1955). These entozoic amoebae differ considerably from one another in their nuclear structure, and each has a characteristic process of nuclear division. It will be shown that the process of nuclear division in *I. bütschlii* differs markedly from that of the other five species, especially in the prophase and anaphase stages.

## MATERIALS AND METHODS

Two mushy stool specimens were obtained without purgation on separate days from a 46-year-old woman, who had lived in India and Ceylon for about 6 years. Trophozoites and cysts of *I. bütschlii* were numerous in both specimens. A few trophozoites and cysts of a small race of *Entamoeba histolytica* and of *Endolimax nana* were also present. The large numbers of trophozoites of *I. bütschlii* provided excellent material for the study of nuclear division.

Approximately 150 faecal smears were stained for this study; these were prepared within 3 hr. after the stools were passed. Schaudinn's fluid containing 5% glacial acetic acid gave the most satisfactory fixation. Schaudinn's fluid containing 2% glacial acetic acid (Wenrich, 1937) was also tried but did not give as good results. Fixation was carried out for approximately 3 hr. at room temperature (*c.* 25° C.). The excess mercuric salt was removed by immersing smears for 5 min. in 0·5% iodine dissolved in 50% ethanol. Smears were then stored in 83% (by volume) ethanol at 5° C. until staining was carried out.

Although Delafield's and Lillie-Mayer's haemalum and Gomori's trichrome stain (Lillie, 1954) were also tried, Heidenhain's iron alum-haematoxylin was preferred for sharpness and clarity in revealing the nuclear structures. The ensuing description of nuclear division in this study is primarily based, therefore, on material stained with Heidenhain's iron alum-haematoxylin without counter-stain for cytoplasm. Smears were mordanted in 4% aqueous iron alum solution for about 5 hr. and then stained in 0·5% haematoxylin (5% alcoholic haematoxylin 1 part, plus distilled water 9 parts) for approximately 16 hr. at room temperature (*c.* 25° C.). 1 or 2% aqueous iron alum solution (prepared from 4% stock kept at 5° C.) was used for de-staining; this was controlled microscopically at × 430 magnification. After de-staining was completed, each smear was rinsed in distilled water and exposed to ammonia fumes for a few seconds or immersed in 0·5% aqueous lithium carbonate solution for 1 min. After washing in gently running tap water for 30 min., smears were dehydrated in graded ethanol (70, 95 and 100%), cleared in four changes of xylol and mounted in gum dammar (dissolved in xylol).

## DESCRIPTION

*Interphase.* Since the interphase stage usually predominates in a population of normal organisms, the majority of published descriptions of nuclear structure of *I. bütschlii* undoubtedly are of resting nuclei. Those given by Dobell (1919), Taliaferro & Becker (1922) and Wenrich (1937) are obviously based on interphase nuclei. Most of the trophozoites of *I. bütschlii* we studied possessed nuclear structures similar to those described by Wenrich (1937, figs. 30, 31, 34, 35, 36) and are regarded as organisms at interphase stages. This view is further supported by our studies of nuclear structures at telophase stages and in organisms undergoing cytokinesis.

The interphase nucleus of the trophozoite of *I. bütschlii* is vesicular and is enveloped by a definite nuclear membrane. Peripheral chromatin granules, similar to those characteristically seen in the genus *Entamoeba* and also frequently seen in the genus *Endolimax*, were not observed attached to the nuclear membrane except on rare occasions, as shown in Pl. I, fig. 5. Present within the nuclear membrane —and usually located centrally—is a deeply and uniformly stained large endosome with a smooth outline. An exception to this common picture is shown in Pl. I, fig. 4, in which the endosome is presented as a thick ring containing a central granule. The zone (periendosomal) between the endosome and the nuclear membrane is occupied by a number of granules (periendosomal granules) which are connected to the endosome by fine fibrils. The periendosomal granules are usually arranged in

a single row, but occasionally occur in two or three rows. At times the periendosomal granules are disposed so close to the periphery that they appear to be located on the nuclear membrane, but they are always connected to the endosome by fine fibrils (Pl. VIII, fig. 5). These granules may occasionally be scattered throughout the entire zone but frequently occur only within a small area. In a small number of trophozoites, no periendosomal granules could be recognized. When they are not observed, it does not necessarily mean that they are absent, but, rather, may indicate superimposition on the endosome.

Table 1. *Relative frequency of four types of normal interphase nuclei in the trophozoite of* Iodamoeba bütschlii*

| Slide no. | Type I (less than 25 %) | Type II (25–50 %) | Type III (50–75 %) | Type IV (over 75 %) | Irregular type | Total |
|---|---|---|---|---|---|---|
| 836 | 17 | 35 | 30 | 15 | 3 | 100 |
| 778 | 10 | 35 | 36 | 16 | 3 | 100 |
| 703 | 13 | 39 | 25 | 20 | 3 | 100 |
| 718 | 5 | 39 | 33 | 19 | 4 | 100 |
| 707 | 9 | 36 | 41 | 13 | 1 | 100 |
| 858 | 17 | 30 | 38 | 12 | 3 | 100 |
| 878 | 15 | 29 | 36 | 17 | 3 | 100 |
| 788 | 20 | 31 | 33 | 15 | 1 | 100 |
| 779 | 16 | 34 | 32 | 15 | 3 | 100 |
| 781 | 21 | 38 | 28 | 11 | 2 | 100 |
| Total | 143 | 346 | 332 | 153 | 26 | 1000 |
| % | 14·3 | 34·6 | 33·2 | 15·3 | 2·6 | |

* As observed in counts of 100 nuclei per slide.

In order to study the relative frequency of various forms, the interphase nucleus was arbitrarily classified into four types, depending on the area of the periendosomal zone that was occupied by periendosomal granules. Allocated to type I, (Pl. VIII, fig. 1), were nuclei wherein less than 25 % of the periendosomal zone was occupied by the periendosomal granules; types II (Pl. VIII, fig. 2), III (Pl. VIII, fig. 3), and IV (Pl. VIII, figs. 4–7), had 25–50, 50–75 and over 75 %, respectively, of the periendosomal zone occupied by the granules. Table 1 shows the distribution as to morphological type of 1000 trophozoites with interphase nuclei. Type I nuclei were present in 14·3 %, type II nuclei in 34·6 %, type III nuclei in 33·2 % and type IV nuclei in 15·3 %. The irregular type constituted only 2·6 % and represented those nuclei which appeared either degenerate or abnormal. Types II and III nuclei were the most prevalent forms, and together composed over two-thirds of the total.

In well-de-stained preparations, occasionally the endosomes of type IV nuclei (and less frequently those of type III nuclei) appear as several darkly stained patches on a lighter background (Pl. VIII, figs. 3, 7). They closely resemble prophase endosomes except that the periendosomal granules are clearly differentiated. It seems probable that interphase nuclei undergo progressive development from types I to IV and finally are transformed into prophase stages.

*Prophase.* The prophase changes are apparently initiated by incorporation of periendosomal granules into the endosome, and at the same time reorganization of the endosome begins. The endosome enlarges considerably, and its central zone becomes less intensely stained. As the periendosomal granules become attached to the endosome, the usually round or oval endosome appears irregularly angular (Pl. VIII, fig. 8). After the granules disappear completely into the enlarging endosome, it becomes rounded again and the margin extends to the area formerly occupied by the granules (Pl. VIII, fig. 9). In the more advanced prophase stages, the endosome appears to be composed of a number of granular bodies embedded in a less intensely stained endosomal matrix. At one stage of prophase more than ten coarse granules are present (Pl. VIII, fig. 10), and at other stages more than twenty fine granules have been noted in the reorganizing endosomes (Pl. VIII, fig. 11). The fine granules, as depicted in fig. 11, approximate in size to the chromosome granules seen in the metaphase and anaphase stages. In later prophase stages, the endosome changes in shape to a stumpy spindle with each pole occasionally containing a dot (Pl. IX, figs. 12–14). A centrodesmus may also be seen at this time (Pl. IX, fig. 13), but it is usually more clearly discernible at the metaphase, anaphase and telophase stages (Pl. IX, figs. 21,22; Pl. X, figs. 24, 25). By the end of prophase, the granular bodies in the endosome (chromosome granules) move toward the equatorial zone of the spindle which is well formed but which does not as yet show clear spindle fibres (Pl. IX, fig. 15).

*Metaphase.* One of the earliest metaphase stages seen is shown in Pl. IX, fig. 16. Here the chromosome granules are arranged at the equatorial zone and appear as an undifferentiated darkly stained mass. However, the spindle fibres are clearly recognizable. At each pole a division centre, appearing as a darkly stained dot, is quite regularly present at this and at later stages. Somewhat later the two sets of daughter chromosomes separate, but each set is still arranged on the same plane (Pl. IX, figs. 17–19). Thus, the arrangement of daughter chromosomes at metaphase differs from that at anaphase wherein the arrangement is in an irregular manner. The chromosome granules appear elongate, lying along the spindle fibres, and are more or less separated. Although the number of chromosome granules could not be determined with certainty, usually more than ten—possibly twelve—granules in each set could be distinguished. The poles of the spindle are usually in contact with the nuclear membrane which appears distended and assumes the shape of the spindle. A polar cap was not observed in any of the stages of nuclear division.

*Anaphase.* The earliest anaphase stage observed is illustrated in Pl. IX, fig. 20. The chromosome granules are longer and more slender than at the metaphase stages; the spindle is also more elongate and appears to cause greater distension of the nuclear membrane. The movement of the daughter chromosomes toward the poles is not synchronous, and they are usually seen lying in several planes (Pl. IX, figs. 20–22). In several trophozoites the centrodesmus appeared to extend beyond the nuclear boundary (Pl. IX, figs. 21, 22) and to contain a granule at each extremity (Pl. IX, fig. 21). The nuclear membrane remained clearly defined in most instances, but in a few amoebae part of the nuclear membrane appeared interrupted by vacuoles (Pl. IX, fig. 20).

*Telophase.* Reorganization of the endosomes from the daughter chromosomes usually takes place as each set of daughter chromosomes approaches the poles of the spindle (Pl. X, figs. 23–25). However, the periendosomal granules usually reappear at a later stage when the newly formed daughter nuclei are about to separate (Pl. X, figs. 26–31). The daughter chromosomes in each set coalesce to form an amorphous mass which stains uniformly dark. Spindle fibres radiating from the poles are usually obscured by the reorganizing daughter chromosomes, but a few spindle fibres and the centrodesmus can usually be seen between the two daughter endosomes (Pl. X, figs. 24, 25). In the amoeba pictured in fig. 23, both sets of coalescing daughter chromosomes are very closely arranged and are still at some distance from the poles; spindle fibres can be seen to extend from the poles into the condensing daughter chromosomes. It is considered that this organism was in a very early telophase stage and was slightly overstained. The early stage of separation of the daughter nuclei was not observed. However, in the later phase of separation the dividing nucleus becomes constricted in the middle. The constriction finally severs the parent nucleus, but the daughter nuclei thus formed are usually connected for some time with a thin strand which includes the centrodesmus (Pl. X, figs. 26, 27). The daughter nuclei are pear-shaped at this stage but may become rounded after the connecting strand is broken off (Pl. X, (figs. 26–30). Periendosomal granules also reappear at this period (Pl. X, figs. 26, 28, 29). The periendosomal granules in the daughter nucleus appear to be cast off from the reorganizing endosome as it condenses to form a deeply and uniformly stained body (Pl. X, figs. 28–30). In general, the number of periendosomal granules which appear at late telophase stages are few, occupy only a small area of the periendosomal zone, and are similar to those of types I and II interphase nuclei.

*Cytokinesis.* When the newly formed daughter nuclei pull away from each other trailing the centrodesmus between them, they usually move toward opposite poles of the elongating cytoplasm (Pl. X, fig. 27). Finally, the cytoplasm constricts in the middle and the counterparts separate, each containing a daughter nucleus (Pl. X, fig. 31). In a freshly separated daughter cell, the nucleus may retain the trailing tail and appear tadpole-shaped. The periendosomal granules and the endosome usually return to the condition of type I interphase nuclei (Pl. X, fig. 32). The nuclear membrane is present throughout the process of nuclear division in this organism.

As can be noted in the figures, the majority of the trophozoites contain various cytoplasmic inclusions, mostly bacteria and yeast cells, in numerous vacuoles. The vacuoles frequently fill the cytoplasm and are a characteristic feature of *Iodamoeba.*

## DISCUSSION

Since a small race of *Entamoeba histolytica* and *Endolimax nana* were also present in the stained material used for this study, it is desirable to emphasize that the description of nuclear division here recorded has reference only to *Iodamoeba bütschlii.* The trophozoites of *I. bütschlii* were far more abundant in the preparations than those of *E. histolytica* and *E. nana,* the approximate ratios being 30:2:1

respectively. The average size of fifty trophozoites of *I. bütschlii* (including the thirty-two organisms used for drawings) was $13{\cdot}7\mu$ with a standard deviation of $\pm 2{\cdot}2\mu$, while the average sizes and standard deviations of fifty trophozoites of *E. histolytica* and *E. nana* at interphase stages were $9{\cdot}2 \pm 1{\cdot}5\mu$ and $8{\cdot}8 \pm 1{\cdot}3\mu$ respectively. Statistically, the differences in size between *I. bütschlii* and *E. histolytica*, and *I. bütschlii* and *E. nana* are significant. In addition to the usual morphological differences, specific differences in the process of nuclear division between *I. bütschlii* and the other entozoic amoebae were noted.

Although the structures of interphase nuclei in *I. bütschlii* were studied extensively by Wenrich (1937), and variation in the periendosomal granules was noted, no attempt was made to classify these nuclei on the basis of the granules. The four types of interphase nuclei in the trophozoites of *I. bütschlii*, as classified in the present study, are apparently connected with the process of nuclear division in this organism.

The nuclear structures of *I. bütschlii* during the prophase stages (Pl. VIII, figs. 8, 9), might possibly be confused with those of *E. nana*, as shown by Dobell (1943, figs. 3, 4). However, in the case of *E. nana*, peripheral chromatin granules are present on the nuclear membrane, while in *I. bütschlii* peripheral chromatin granules (not to be confused with periendosomal chromatin granules) are lacking on the nuclear membrane, and the endosome in relation to nuclear size is larger in *Iodamoeba* than in *E. nana*. At late prophase the endosome of *E. nana* completely disorganizes to form a number of granules which scatter over the entire nucleus, and the chromosomes are formed out of a fraction of these granules (Dobell, 1943, figs. 7–9). In *I. bütschlii* the endosome retains its identity until the spindle is well formed, and the granules developing in the endosome appear to be the chromosomes (Pl. VIII, figs. 10, 11; and Pl. IX, figs. 12–14). It is interesting to note that the periendosomal granules in *I. bütschlii* appear to participate directly in forming the chromosomes as predicted by Wenrich (1937). This is in contrast to *E. histolytica* and *E. coli*, in which peripheral chromatins do not appear to participate directly in forming the chromosomes (Kofoid & Swezy, 1925; Pan & Geiman, 1955).

The metaphase stages in *I. bütschlii* are also different in several aspects from those of other entozoic amoebae of man. During early metaphase, the chromosomes of *I. bütschlii* are arranged at the equatorial zone so closely that they appear as a darkly stained mass, and their number could not be counted (Pl. IX, fig. 16). This appearance has not been observed in the other amoebae. At late metaphase, when chromosomes can be counted with some certainty, there are usually more than ten and possibly twelve chromosomes per daughter set. The chromosome numbers given for other amoebae are: *Endolimax nana*, 10 (Dobell, 1943); *Dientamoeba fragilis*, 4 and 6 (Wenrich, 1939, 1944; Dobell, 1940); *Entamoeba histolytica*, 6 or fewer (Kofoid & Swezy, 1925; Uribe, 1926; Dobell, 1928; Cleveland & Sanders, 1930); *E. coli*, 6–8 (Dobell, 1938); and *E. gingivalis*, 6 (Child, 1926). It would appear that *I. bütschlii* has the largest number of chromosomes among the intestinal amoebae.

The most unusual structure seen during the process of nuclear division in *I. bütschlii* was observed at the anaphase stage, when the centrodesmus appeared

to extend beyond the nuclear boundary, although the nuclear membrane appeared intact (Pl. IX, figs. 21, 22). Although in *D. fragilis* the centrodesmus was shown to distend the nuclear membrane at anaphase (Wenrich, 1939, 1944; Dobell, 1940), extension of the centrodesmus beyond the nuclear boundary has not been described.

The nuclear structures of telophase stages in *I. bütschlii* also resemble those of *E. nana*. However, the nuclear membrane disappears at late anaphase and reappears at late telophase in *E. nana* (Dobell, 1943, figs. 15–18), while in *I. bütschlii* the nuclear membrane does not disappear, the reorganizing daughter endosome reassumes its smooth outline quickly and the periendosomal granules usually reappear at late telophase (Pl. X, figs. 23–29). Since periendosomal granules reappear in the telophase stages gradually, little confusion should arise in recognizing stages later than telophase in *I. bütschlii*, and types I and II nuclei are more closely related to nuclei which have just divided (cf. Pl. VIII, fig. 1 and Pl. X, fig. 32).

It should be clear from the above discussion that the process of nuclear division of *I. bütschlii* as here described is different from that described for the other species of entozoic amoebae in man. The question raised by Brug (1921) as to the validity of genus *Iodamoeba* can definitely be disregarded.

## SUMMARY

1. Study of the nuclear morphology of a large number of haematoxylin-stained specimens of *Iodamoeba bütschlii* has permitted description of the process of nuclear division in this organism.

2. The interphase nuclei of the trophozoites were classified into four types (types I to IV) depending on the distribution of the periendosomal granules. The development of the four types of interphase nuclei appears to follow the process of nuclear division.

3. During the early prophase stages the periendosomal granules are incorporated into the endosome. More than twenty small granules are formed from the organizing endosome.

4. In late metaphase stages more than ten, and probably twelve, granules can usually be counted per set of the daughter chromosomes.

5. The centrodesmus may extend beyond the nuclear boundary during the anaphase stages, while the nuclear membrane appears intact.

6. The periendosomal granules reappear by the end of telophase stages when the two daughter nuclei are nearly completely separated.

7. In the freshly separated daughter cell the nucleus may still retain the trailing tail and appear tadpole-shaped, but the periendosomal granules are usually arranged like those in the type I interphase nucleus.

8. The validity of the genus *Iodamoeba* is re-emphasized on the basis of the characteristic nuclear structures and the unique process of nuclear division as here described for *I. bütschlii*.

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## EXPLANATION OF PLATES VIII–X

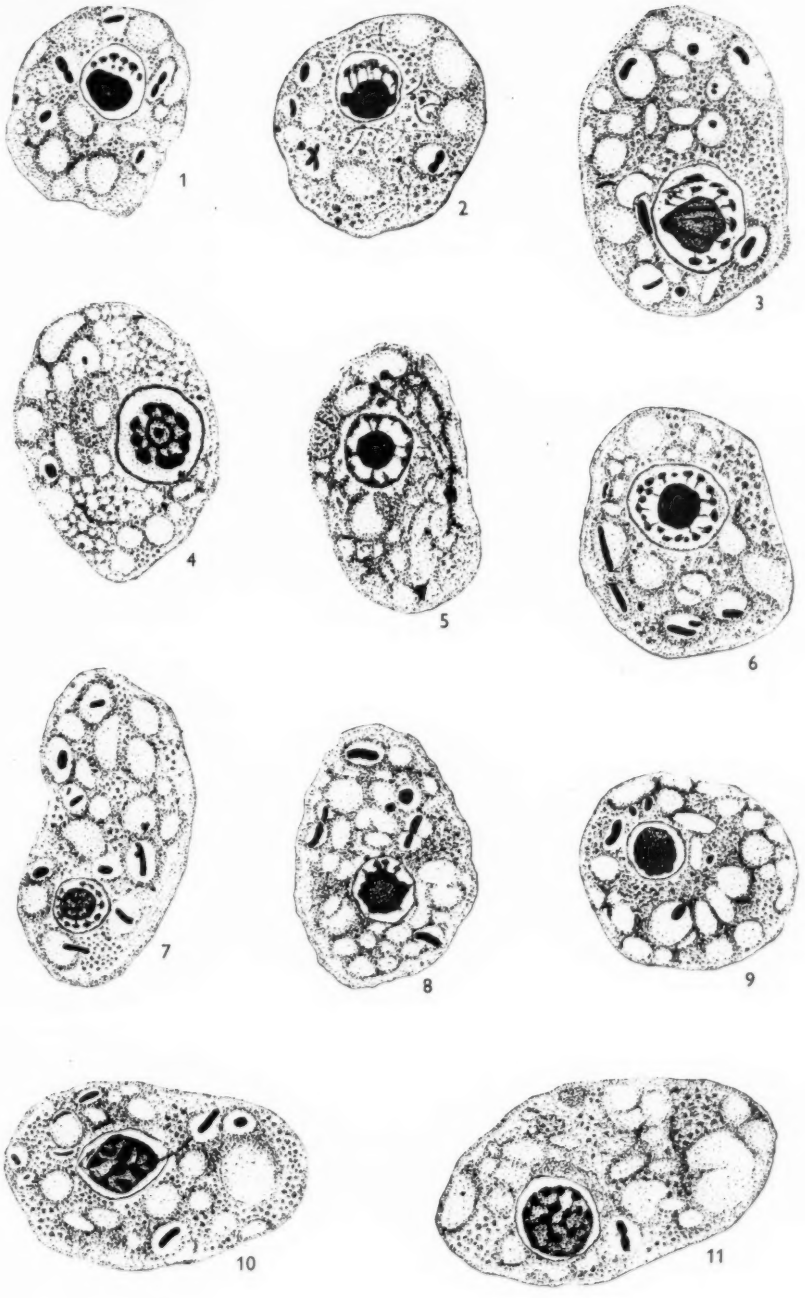
All drawings were made with the aid of a camera lucida from preparations fixed in Schaudinn's fluid containing 5% of glacial acetic acid (only fig. 11 was fixed in Schaudinn's fluid containing 2% of glacial acetic acid) and stained with Heidenhain's iron alum haematoxylin. The original magnification was *ca.* × 3200, and figures were reduced to four-fifths on reproduction.

### PLATE VIII

Fig. 1. Organism with type I interphase nucleus.

Fig. 2. Organism with type II interphase nucleus.

Fig. 3. Organism with type III interphase nucleus. Note the unevenly stained endosome. The trophozoite may have started reorganization of the endosome for nuclear division.

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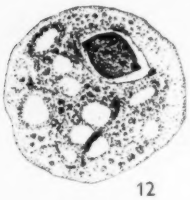
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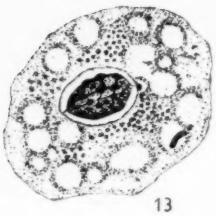
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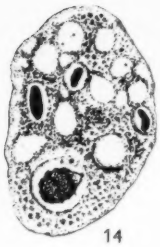
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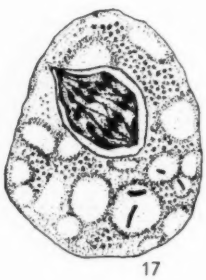
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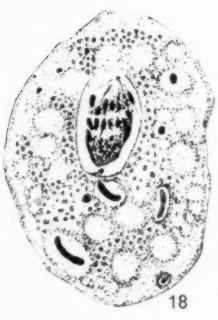
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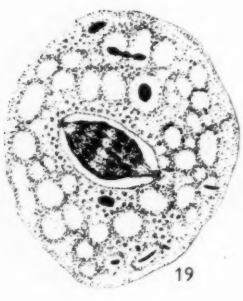
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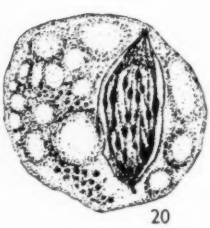
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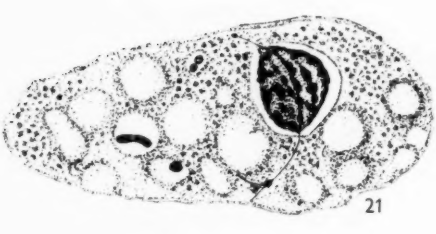
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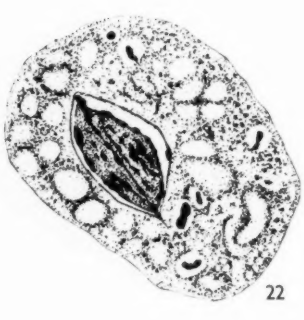
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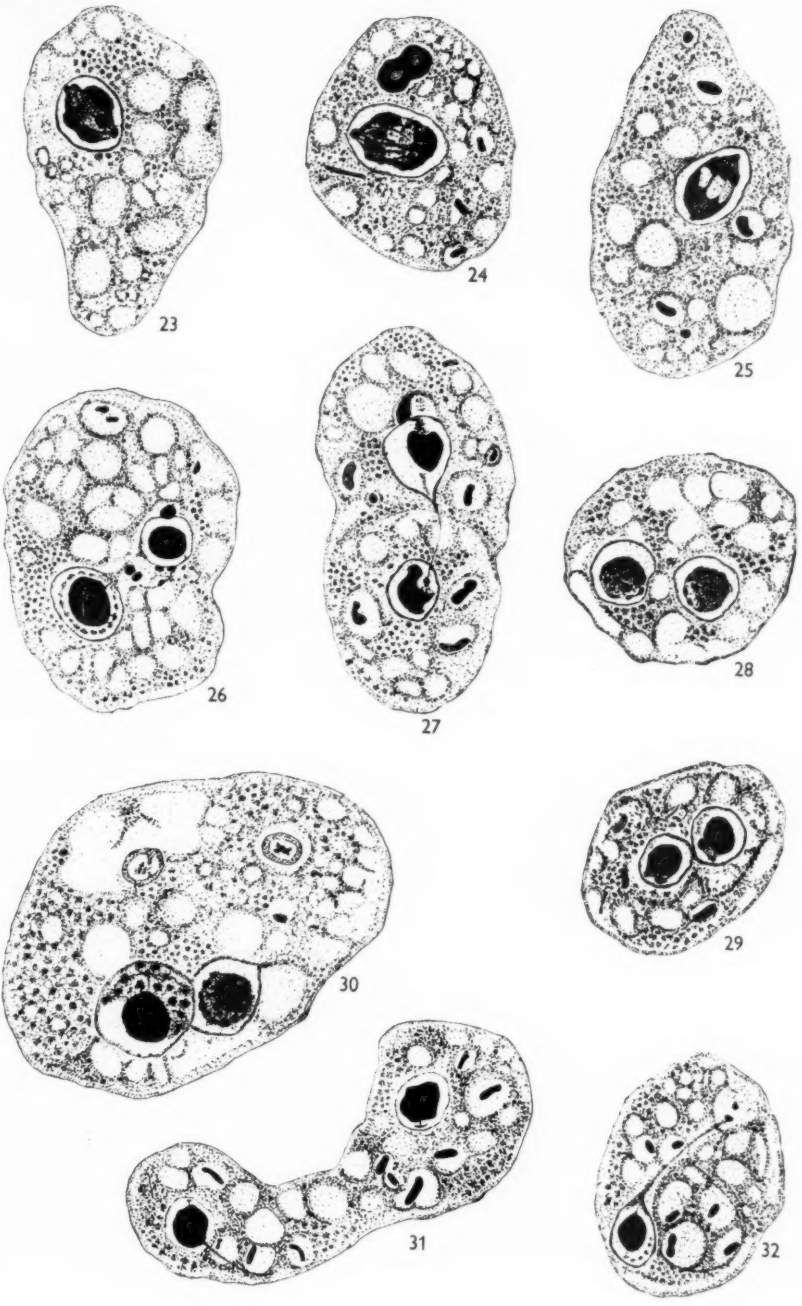
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Fig. 4. Trophozoite with type IV interphase nucleus. The endosome is a thick ring containing a central granule.

Fig. 5. Trophozoites with type IV interphase nucleus. Note the peripherally disposed periendosomal granules which are in contact with the nuclear membrane.

Fig. 6. Organism with a typical type IV interphase nucleus.

Fig. 7. Organism with type IV interphase nucleus. The unevenly stained endosome suggests reorganization of the nucleus for division.

Fig. 8. Trophozoite with early prophase nucleus. Most periendosomal granules have been incorporated into the endosome, which assumes an irregular outline.

Fig. 9. Trophozoite with prophase nucleus. Incorporation of periendosomal granules is nearly complete.

Fig. 10. Organism with prophase nucleus. More than ten coarse granules (chromomeres?) are present in the endosome.

Fig. 11. Trophozoite with prophase nucleus. More than twenty fine granules (chromosomes?) are present in the endosome.

PLATE IX

Fig. 12. Organism with prophase nucleus. Early stage of spindle formation.

Fig. 13. Trophozoite with prophase nucleus. Spindle formation is more complete than fig. 12 and the centrodesmus has appeared.

Fig. 14. Trophozoite with late prophase nucleus. Chromosomes are moving toward the equatorial plate.

Fig. 15. Organism with late prophase nucleus. Chromosomes approach the equatorial plate and the spindle is nearly completed. Spindle fibres are recognizable.

Fig. 16. Trophozoite with early metaphase nucleus. Chromosomes are arranged closely at the equatorial plate and are seen as a darkly stained mass. Spindle fibres are clearly discernible.

Figs. 17, 18. Organisms with late metaphase nuclei. Chromosomes begin to move away from equatorial plate but are still arranged in a plane.

Fig. 19. Trophozoite with nucleus at late metaphase (or early anaphase).

Fig. 20. Trophozoite with anaphase nucleus. Note the large spindle and the arrangement of chromosomes in zig-zag manner.

Figs. 21, 22. Organisms with anaphase nuclei. Note the extension of centrodesmus beyond the nuclear boundary.

PLATE X

Fig. 23. Organism with telophase nucleus.

Figs. 24, 25. Organisms with telophase nuclei. Spindle fibres and centrodesmus are still discernible between two sets of coalescing daughter chromosomes.

Figs. 26, 27. Organisms with nearly completely separated daughter nuclei. Note the more or less pear-shaped daughter nuclei and the connecting strand.

Figs. 28–30. Binucleate organisms with condensing endosome. Periendosomal granules have reappeared.

Fig. 31. Binucleate organism undergoing cytokinesis.

Fig. 32. Freshly separated daughter cell with tadpole-shaped nucleus.

(*MS. received for publication* 23. I. 1959.—Ed.)

# *COLEOSPORA BINUCLEATA*, GEN.NOV., SP.NOV., A HAPLOSPORIDIAN PARASITE FOUND IN *GONOCEPHALUM ARENARIUM* (COLEOPTERA)

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(With 16 Figures in the Text)

The haplosporidian described is found in the Malpighian tubules of the beetle *Gonocephalum arenarium* in the Cape Peninsula, South Africa.

With the exception of the germination, which must take place in the digestive tract, the life cycle is completed within the Malpighian tubules. Studied over a period of several years, it has been found that the incidence of infection averages about 5%.

Six Malpighian tubules connect with the gut, and in an infected insect all six are almost always parasitized to the same degree and by the same forms of the parasite.

An early and striking effect of the haplosporidian on the host tissue is the formation of a number of nipple-like protuberances which project from the external surfaces of the tubule walls.

Apart from an intracellular plasmodial phase of multiplication, which continues almost throughout the course of the infection, the parasites are adherent to the inner surfaces of the tubules, and remain so until the final phase, when the sporocysts become mature.

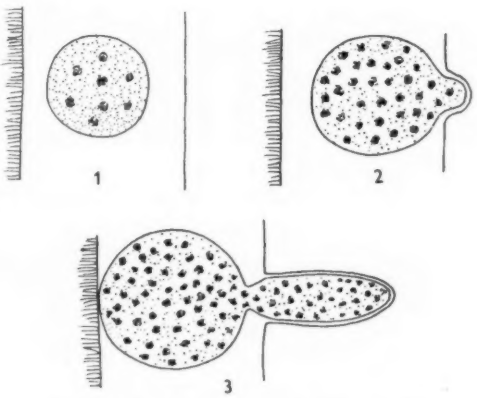
## PRIMARY PLASMODIAL PHASE AND NIPPLE-LIKE SHEATH

The free sporoplasm has not been found, and the development preceding the intracellular plasmodial phase cannot, therefore, be described. In the earliest observed stage of the infection a few spherical, intracellular plasmodia, measuring about 9$\mu$ across, each with some eight nuclei are found within the tissue of the tubule walls (Fig. 1). The number of nuclei increases and the bodies become considerably larger. For a reason which is not at all clear the plasmodium always extends first in the direction of the outer surface of the tubule wall where it forms a bulge but remains invested in a cytoplasmic sheath derived from the host-cells (Fig. 2).

Continued growth of the plasmodium results in the extension of this cytoplasmic sheath until it and the contained plasmodium projects from the exterior surface of the tubule for a distance of about 15$\mu$ in nipple-like form with a maximum breadth of about 7$\mu$. While the protuberance is being formed, that portion of the plasmodium which lies within the tissue of the wall remains bulbous and increases in size. Just beneath the exterior surface of the tubule the plasmodium is always slightly constricted (Fig. 3). Later, the bulbous portion extends into the lumen of the tubule where the whole plasmodium, including that part invested by the

protruding sheath, becomes free. It is not known whether the cells of which it is composed are liberated as individual bodies or whether it merely breaks up into smaller plasmodia. Separate mononucleate cells measuring some 2·5$\mu$ across are always present, but small multinucleate plasmodia predominate at this stage.

In view of the presence of mononucleate cells it is possible that the intracellular plasmodium breaks up into a number of separate cells upon entering the lumen of the tubules, and that these cells then undergo a series of nuclear divisions which are not followed by cytoplasmic cleavage and thus commence a secondary and extracellular plasmodial phase.




Figs. 1–3. For explanation see p. 558.

## FATE OF INVESTING SHEATH

When the primary plasmodium has been liberated into the lumen of the tubule, both the cytoplasmic nipple-like protuberance, which formerly invested a portion of it, and the intracellular cavity which held the bulbous part, maintain their form for a while. The intracellular cavity soon disappears, but the cytoplasmic projection persists for a longer period. It is not unusual, during the later stages of the infection, to find more empty protuberances than those occupied by the parasite (Fig. 4 *a*). While it is difficult to estimate the number of these projections they must, on occasion, be present in numbers considerably in excess of 100 per tubule; it is only near the extremities of the tubules that they are not found. In view of their presence in such large numbers it seems impossible that each could result from the penetration of the wall by a sporoplasm; it seems, rather, that certain forms are produced by the liberated plasmodium which have the ability to re-enter the cells of the tubules and commence the intracellular plasmodial phase afresh.

The protuberances continue to be produced by intracellular plasmodia until a late stage of the infection. Sections of tubules within which sporogony was predominant over the earlier phases continue to show large numbers of them filled by plasmodia. It is only during the final stage in the life cycle of the parasite, when the tubules are packed, almost to the complete exclusion of other forms, with mature, separated sporocysts, that the nipples may be almost or entirely absent.

## SECONDARY PLASMODIAL PHASE

The forms found within the lumen of the Malpighian tubules prior to the beginning of the sexual phase are mainly multinucleate plasmodia measuring about $9\mu$ across when spherical, and which rarely possess more than 8 nuclei (Fig. 4*b*). As previously stated a few small mononucleate cells measuring about $2{\cdot}5\mu$ across are also found, together with larger forms containing 2, 3 or any number up to the maximum number of nuclei found at this stage.

The plasmodia are attached to the inner surface of the tubule walls by means of pseudopodial, cytoplasmic processes. These processes are not observed among the mononucleate cells or forms with very few nuclei. As the infection increases in intensity the plasmodia become larger and contain more nuclei. Just prior to the commencement of the sexual phase it is not unusual to find forms with over 20 nuclei.

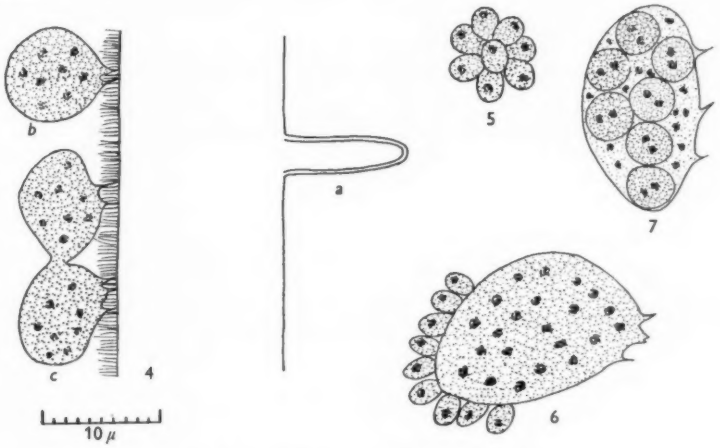
The plasmodia cannot be described as having any characteristic shape. They may be spherical or completely irregular in outline, or elongate with a constriction in the middle of the body. This last shape is only observed among those with the larger number of nuclei, and it appears very likely that the plasmodium divides into two smaller bodies (Fig. 4*c*). When studied in the fresh state within the tubule or in sections it is found that the plasmodium always elongates in a direction parallel to the tubule.

## GAMETOGONY AND SPOROGONY

When the secondary plasmodial phase has progressed to the stage when large portions of the tubules become heavily involved, and many larger plasmodia are present having 20 or more nuclei, sexual phenomena begin to occur between adjacent pairs of cells within the larger multinucleate bodies. It has not been found possible to establish definitely whether sexual fusion takes place between adjacent cells of the same generation produced within the plasmodium, or whether cells of another type, which appear at this time, are involved in the phenomenon. The latter cells differ from those making up the plasmodial bodies in that cytoplasmic cleavage, although not complete, follows nuclear division and results in the formation of small cell clusters. These cells, which measure some $2{\cdot}5\mu$ across, remain connected at a point diametrically opposite to the nuclei, which are eccentrically situated. Multiplication is by binary fission and it is not uncommon to find one or more of the group undergoing division. A series of such divisions results in a group which, in dry-fixed preparations, presents a rosette-like formation. In the fresh state, or when wet-fixed, they do not lie in the same plane and present the appearance, rather, of a small bunch of grapes, which is rarely made up of more than 12 cells (Fig. 5).

Groups of such cells are frequently observed adhering to the surface of a plasmodium (Fig. 6) and occasionally appear to be almost completely merged within that body. It appears possible, therefore, that sexual fusion takes place between the cytoplasmically undifferentiated cells of the plasmodium and groups of others which become diffused within the plasmodium, and which are then not visibly differentiated in any way from the rest of the multinucleate body.

Only about one-half of the cells comprising a plasmodium take part in the sexual process which is initiated by the cytoplasmic fusion of adjacent gametes. Immediately after fusion, and before syngamy, the cytoplasm of each pair undergoes a striking change and becomes sharply differentiated from that associated with nuclei which are not involved. The paired gametes form spherical, binucleate cells measuring some 3·5$\mu$ across, the cytoplasm of which takes on a deep, smooth blue when stained by Giemsa's method, in contrast to the residual portion, which stains delicately and is frothy in nature (Fig. 7). In wet-fixed and stained preparations the fused pairs appear to lie within vacuoles slightly larger than themselves in the plasmodium and each contains two vesicular nuclei with central karyosomes (Fig. 8). The paired gametes are also clearly visible within the plasmodium in the fresh state. In the fresh state, also, it can readily be observed that, soon after the fusion of the gametes, the whole group becomes invested by a membrane.




Figs. 4–7. For explanation see p. 558.

The number of paired gametes formed within any group is usually from five to ten. The nuclei which do not take part in the sexual process remain readily demonstrable within the group until the final maturation and separation of the sporocysts. When wet-fixed and stained, these residual nuclei always appear as a pair of parallel rods of chromatin.

The cytoplasmic fusion of the gametes, which has been described above, the syngamy which follows, and all subsequent stages in the formation and maturation of the sporocyst, are almost perfectly synchronized within each group. First the nuclei of each of the cytoplasmically fused pairs approach each other and syngamy occurs simultaneously, resulting in the formation of from 5 to 10 round zygotes in which the synkaryon is eccentrically situated (Fig. 9). Later, the zygotes elongate until they measure some 11·5$\mu$ long with a maximum breadth of about 4·5$\mu$. The nuclei of these sporoblasts, as they by now have become, are centrally situated. They lie in a generally parallel orientation and the unit increases in size

and takes on ovoid form (Fig. 10). Each sporoblast then commences to secrete a cyst wall.

When the cyst wall is fairly well developed the nucleus undergoes one division, each daughter nucleus being located about one-third of the body-length from the ends of the sporoplasm (Fig. 11). Cytoplasmic cleavage has never been found to follow this nuclear division, the binucleate sporoplasm being the final stage seen.

Throughout the development of the group of sporocysts they remain invested by the membrane. The residual cytoplasm of the plasmodium disintegrates soon after the zygotes begin to elongate, but the residual nuclei become attached to the surfaces of the sporocysts, where they remain apparent until the sporocysts finally separate, after which they can no longer be found. When the group is thoroughly mature, the enveloping membrane disappears and the sporocysts are released within the lumen of the tubule.

In the final stages the tubules become packed with almost mature groups and with free, detached sporocysts, and it is found that the tubules actually broaden in order to accommodate the mass of parasites.

Sporocysts are found in the faeces of the host but only in small numbers, and it is felt that infection by the method of faecal contamination is greatly supplemented by the dissemination of sporocysts after the death and disintegration of the host.

## THE SPOROCYST

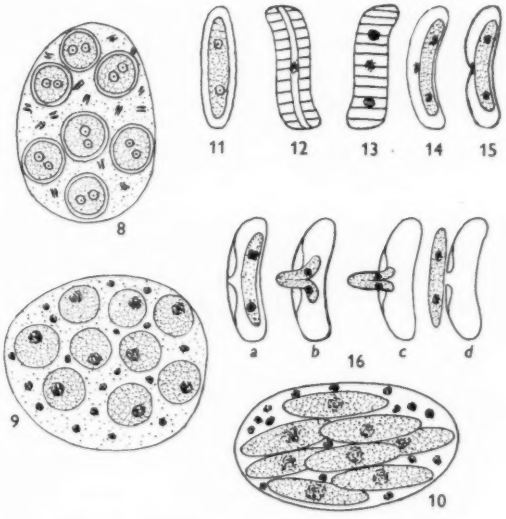
The sporocyst is a complex structure, measuring about 10·5$\mu$ in length and about 2·5$\mu$ in maximum breadth. Its most common appearance when lying in saline is shown in Fig. 12. In this orientation it presents a bi-valved appearance owing to the presence of two elevated, marginal ridges, while the extreme ends of the body are slightly curved in opposite directions. Each of the ridges exhibits about twelve corrugations which lie at a slightly oblique angle to the long axis of the sporocyst. The corrugations do not appear to extend into the depression running down the middle of the body. At the centre there is a dark, round area, visible in the fresh state, which often stains intensely by Giemsa's stain or by Ehrlich's haematoxylin.

The opposite surface of the cyst (the undermost according to the previous orientation), is shown in Fig. 13, which is from a specimen stained by Ehrlich's haematoxylin. This surface is unbroken by any depression and the corrugations, also about twelve in number, extend obliquely from side to side.

When 'lying on its side', as it were, the sporocyst is curved as shown in Fig. 14, which represents a stained specimen as seen at either the lowest or highest level of focus, both of which present the same picture. Focused at the middle level and showing the outline of the central depression, the sporocyst appears as at Fig. 15. Here, the contour of the wall dips sharply towards the middle, and at the point of deepest depression is located the dark-staining structure which, in the first-described orientation, appeared as a round area. This structure seems to be almost in contact with the middle of the sporoplasm and is practically certainly in the nature of an absorbable or dissolvable 'plug' which allows the escape of the sporoplasm under favourable conditions.

### EMERGENCE OF SPOROPLASM

No method has been discovered of inducing the emergence of the living sporoplasm from the sporocyst, but it has been found that the sporoplasms are often released, and probably in a manner similar to that occurring in nature, if sporocysts are immersed in a 50 % or stronger solution of nitric acid. A previous period of desiccation lasting some days is necessary to bring about this phenomenon. The reaction may be practically immediate and the sporoplasm almost shot out, or it may take place only after an immersion lasting a quarter of an hour, and require several minutes to emerge completely. In many cases the reaction does not take place at all. When it takes place slowly it is found that, prior to expulsion, a distinct channel can be made out at the centre of the convex side of the sporocyst (Fig. 16*a*), and it is at this point that the sporoplasm emerges in a folded condition (Fig. 16*b*, *c*). After the removal of the acid, the cytoplasm and the two nuclei of the sporoplasm are strongly coloured by Giemsa's stain (Fig. 16*d*).




Figs. 8–16. For explanation see p. 558.

### DISCUSSION

It seems almost certain that the mature sporoplasm is binucleate, and that it emerges as a binucleate, elongated body when germination takes place under natural conditions.

It is difficult to understand why the six Malpighian tubules are nearly always infected simultaneously, unless multiplication of the parasite takes place in the gut prior to the invasion of the tubules, but this has not been observed. It is possible that such a phase may take place during the larval stage of the host but, unfortunately, it has not been found possible to obtain larvae for study.

Interesting and equally difficult to understand is the apparent wastage of gametes within the plasmodium unless, as suggested, the number which become sexually associated is controlled by the number of gametes of opposite sex which become diffused throughout the plasmodium.

The immediate and definite change in the character and staining reactions of the cytoplasm following cytoplasmic fusion of the gametes appears to be almost unique. It seems very likely that the fused cells, even before syngamy, draw from the surrounding cytoplasm substances which bring about this differentiation, following which the cytoplasmic residuum of the plasmodium rapidly disintegrates.

The organism qualifies for inclusion in the Order Haplosporidia in view of the long plasmodial phases and the ridged formation of the sporocyst. As it does not qualify for inclusion in any of the accepted genera the name *Coleospora binucleata* gen.nov., sp.nov. is proposed.

## DEFINITION OF NEW GENUS

Sporoplasm binucleate and elongate. Two phases of plasmodial reproduction, the first intracellular, the second extracellular. Gametogony within plasmodium. Five to ten zygotes are produced, leaving much residual cytoplasm and many residual nuclei. Each zygote produces one sporocyst. Sporocysts elongate, slightly S-shaped or curved according to orientation and corrugated throughout full length. Produces one sporoplasm.

## EXPLANATION OF FIGURES

*Note.* Figs. 1–7, 9, 10 and 16*d* are from dry-fixed and stained preparations. Other figures, excepting those representing the organism in the fresh state (Figs. 12, 16*a*, *b*, *c*) wet-fixed and stained by Ehrlich's haematoxylin. Figs. 1–4 are semi-diagrammatic.

Fig. 1. Primary plasmodium within Malpighian tubule wall.

Fig. 2. Primary plasmodium showing development of protuberance.

Fig. 3. Fully grown primary plasmodium with protuberance.

Fig. 4. (*a*) Empty protuberance after disappearance of intracellular cavity. (*b*) Anchored secondary plasmodium. (*c*) Apparently dividing plasmodium.

Fig. 5. Group of cells which possibly associate sexually with those forming secondary plasmodia.

Fig. 6. Group of same cells (Fig. 5) adhering around periphery of plasmodium.

Fig. 7. Cytoplasmically fused gametes prior to syngamy.

Fig. 8. As Fig. 7, but wet-fixed and stained with Ehrlich's haematoxylin.

Fig. 9. Group containing 9 zygotes.

Fig. 10. Group of elongate sporoblasts.

Fig. 11. Binucleate sporoplasm showing developing cyst-wall.

Fig. 12. Sporocyst. Fresh state.

Fig. 13. Reverse view of sporocyst.

Fig. 14. Side view of sporocyst.

Fig. 15. Side view of sporocyst showing central depression.

Fig. 16. (*a*) Appearance of sporocyst after immersion in 50% nitric acid, showing central channel. (*b*) and (*c*) Emerging sporoplasm. (*d*) Free sporoplasm.

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# A CYTOLOGICAL STUDY OF THE EARLY OOCYSTS OF SEVEN SPECIES OF *PLASMODIUM* AND THE OCCURRENCE OF POST-ZYGOTIC MEIOSIS

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(With 137 Figures in the Text)

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## 1. INTRODUCTION

Our present knowledge of the meiotic division in the genus *Plasmodium* is based on the following two conceptions.

(i) The early conception of the separation of a reduction body from the macrogametocyte prior to fertilization indicating a pregametic meiosis in malaria parasites.

(ii) The second more recent view suggests the occurrence of post-zygotic meiosis. This hypothesis has been derived from observations on the chromosome cycles of coccidia and gregarines.

The early conception received a great deal of attention from many investigators who expected to find pregametic meiosis or reduction division after the fashion of metazoa. Bastianelli & Bignami (1900) first suggested that this might occur in malaria parasites. Schaudinn (1902) observed similar reduction bodies in the preparations of mid-gut contents of fully engorged mosquitoes fed on a case of tertian malaria. Ivanie (1935–37) described two stages of nuclear division during the schizogony of *P. vivax*, an early primitive promitotic type of division which later changed into a true mitosis.

Chen (1944) studied the nuclear division in schizonts of *P. elongatum* using Feulgen's reaction. He described a diffused chromatin mass which stained pink and contained purple bands, which he thought might be chromosomes.

The appearance of paired chromosome-like structures in the rounded microgametocytes of *P. falciparum*, prior to the extrusion of flagella, was observed by MacDougall (1947) and Lüdicke & Piekarski (1952). It was not clear, however, whether the two chromatin masses which entered each flagellum could be considered as two or four chromosomes. Further, these workers drew attention to the formation and separation of a Feulgen-positive reduction body from the macrogametocyte containing thread-like chromatin.

The evidence regarding the second view, that of post-zygotic meiosis, has been derived from observations made by Dobell & Jameson (1915), Jameson (1920) and Dobell (1925), on the chromosome cycle of the gregarine, *Diplocystis schneideri*, and of the coccidian, *Aggregata eberthi*.

Further, the zygotic meiosis had been demonstrated by Naville in *Klossia helicina* (1927*a*), and in *Mattesia dispora* (1930), by Hauschka in *Adelina deronis* (1943), by Phillips & MacKinnon in *Apolocystis elongata* (1946), and by Cleveland in *Trichonympha* (1949). These workers showed that in these organisms the nucleus is diploid only in the zygote state.

Bishop (1955) reviewed the previous work on gametogenesis in Haemosporidiidea and suggested a possibility of the occurrence of post-zygotic meiosis in the genus *Plasmodium*. Recently, Wolcott (1954, 1955, 1957) observed the morphology and number of chromosomes in the asexual forms of four human species of *Plasmodium* and also in *P. knowlesi*, *P. lophurae*, *P. relictum*, *P. floridense* and *P. berghei*. He observed chromosomes during different stages of mitosis in living malaria parasites, using phase contrast microscopy. He suggested that the genus *Plasmodium* has a complex of two chromosomes.

The most comprehensive account of the cytological study of the different stages in the sporogonic cycle of *P. falciparum* was given by Grassi (1900). He described the nuclear division in 2- to 3-day-old oocysts as direct or amitotic.

As far as the literature on malariology is concerned no step has yet been taken to solve the intricate problem of post-zygotic meiosis in the genus *Plasmodium*. The present studies were undertaken in an attempt to find the first nuclear reduction division in the early oocysts of available species of *Plasmodium*, and it is hoped that the findings may help to fill the gap in our knowledge regarding the haploid and diploid cycles of chromosomes in their nuclei.

## II. MATERIALS AND METHODS

Seven species of malaria parasites were used and their appropriate insect vectors. The mosquitoes were deprived of food and water for 48 hr. before an infective blood meal. The laboratory-bred *Aëdes aegypti* var. *queenslandensis* were fed on chicks infected with *Plasmodium gallinaceum* on the fourth day of patency. On this day the mature gametocytes were qualitatively and quantitatively adequate to cause a good infection in these mosquitoes. The other species of anopheline mosquitoes were fed on infected vertebrate hosts such as rhesus monkeys and hamsters using methods similar to those described by Garnham (1951*a*) and Garnham & Lainson (1957). The following species of parasites and vectors and their suitable vertebrate hosts were employed during the cytological study of the early oocysts.

| Vertebrate host | Parasite | Vector |
|---|---|---|
| 1. *Gallus domesticus* | *P. gallinaceum* | *Aëdes aegypti* var. *queenslandensis* |
| 2. Hamsters (*Cricetus frumentarius*) | *P. berghei* | *Anopheles aztecus* |
| 3. Rhesus monkey (*Macacus rhesus*) | *P. cynomolgi* | *A. maculipennis* var. *atroparvus* |
| 4. Rhesus monkey (*Macacus rhesus*) | *P. gonderi* | *A. aztecus* |
| 5. Rhesus monkey (*Macacus rhesus*) | *P. inui* | *A. aztecus* |
| 6. Rhesus monkey (*Macacus rhesus*) | *P. knowlesi* | *A. aztecus* |
| 7. Man | *P. vivax* | *A. stephensi* |

Exflagellation was observed in a drop of drawn infected blood covered with a cover-slip to judge the maturity of microgametocytes prior to the experimental feeding of mosquitoes. The fully engorged females were kept, in the manner described by Garnham (1951*a*), in Lumsden's tubes at a temperature of 28° C., and relative humidity 60–65 %.

The cytological study of the early stages was based on the following methods.

(1) Preparations of thick and thin films of the mid-gut contents (for the observations of zygotes and ookinetes).

(2) Preparations of gently flattened and slightly crushed mid-guts from infected mosquitoes.

(3) Serial, longitudinal sections, 4–5$\mu$ thick, of mid-guts from infected mosquitoes. Carnoy's fluid or 10 % formal saline proved to be more effective fixatives than Schaudinn's fluid for the infected mid-guts, which were dissected out at hourly intervals from 40 to 72 hr. after the blood meal. The mid-guts were fixed

for 1–2 hr. and either lightly stained with Erhlich's haematoxylin or mounted on small, thin, square pieces of brain tissue prior to the ordinary wax-embedding method. The following standard techniques were employed for staining the above-mentioned preparations.

(1) Wolcott's technique (1952).

(2) Iron haematoxylin (Heidenhain's).

(3) Feulgen reaction (Coleman's method, 1938) counterstained with light green.

(4) Aceto-carmine technique specially devised for the study of chromosomes by Darlington & Lacour (1947). This technique did not give satisfactory results.

The serial longitudinal sections of mid-guts stained with the above-mentioned techniques gave unsatisfactory and confusing results. The crushed smears of entire, infected mid-guts, or gently flattened mid-guts, however, produced satisfactory and successful results giving a clear picture of the entire young oocysts, showing the arrangement of chromosomes.

The infected mid-guts were flattened in the manner described below.

The mid-gut was placed on the middle of a clean slide in a small quantity of normal saline and then very carefully and gently flattened or slightly crushed with the edge of a clean cover-slip and immediately fixed in osmic-acid vapour for 30 sec. The rest of the procedure was similar to that described by Wolcott (1952) with the following modifications:

(i) Stained in Giemsa's solution for 10–12 hr. instead of 1 hr. as given in the original technique.

(ii) Hydrolysis in N/1 HCl for 30–45 min.

(iii) Mounted in Green Euparal.

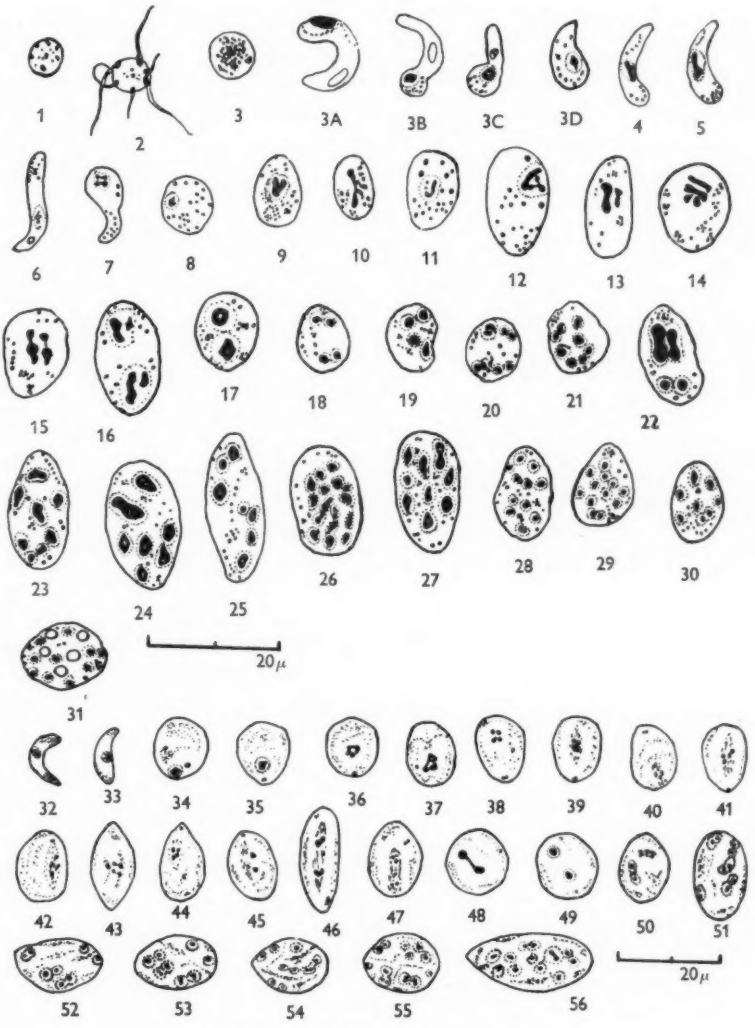
## III. A CYTOLOGICAL STUDY OF ZYGOTES AND OOKINETES OF THE FOLLOWING SEVEN SPECIES OF *PLASMODIUM*:

1. *P. gallinaceum*
2. *P. berghei*
3. *Pl cynomolgi*
4. *P. gonderi*
5. *P. inui*
6. *P. knowlesi*
7. *P. vivax*

Microscopical examination of smears from the mid-gut contents of mosquitoes from 4 to 8 hr. after the infective meal, at half-hourly intervals, showed uninucleate zygotes. The zygotes of *P. gallinaceum* measured 2–3$\mu$ in diameter and contained a deeply staining, central nucleus. The size of the zygotes of other species was approximately 4$\mu$ and they were uninucleate (Figs. 3, 57). The pigment at this stage tended to obscure the nucleus. It is clear from these observations that the fusion of the two gametic nuclei occurred very rapidly in these zygotes.

Dimorphic ookinetes as described by Grassi (1900), Mackerras & Ercole (1948) and Garnham (1951*b*), were also seen in the mid-gut contents from 12 to 24 hr. after the infective meal. The ookinetes observed in the above seven species of *Plasmodium* were uninucleate and the structure of the nucleus was similar to that described by Grassi (1900) except for a peculiar structure of the karyosome in some ookinetes (Figs. 5, 6). These ookinetes showed an irregular outline of a heavily

staining, rhomboid-shaped, beaded mass of chromatin with an unstained, small central area (Fig. 7). This whole mass of chromatin was surrounded by a clear zone representing the nucleoplasm with a distinct nuclear membrane. The dimorphic ookinetes of *P. berghei*, *P. cynomolgi* and *P. gonderi* showed a similar




Figs. 1–56. For legends see p. 581.

nuclear structure with a round mass of chromatin at the resting stage (Figs. 32, 33, 58, 59, 76, 77). Study of the nuclei of the ookinetes therefore indicated that the first nuclear division had not yet started.

## IV. MEIOTIC PHASES IN THE EARLY OOCYSTS OF SEVEN SPECIES OF *PLASMODIUM*

### 1. *Plasmodium gallinaceum*

(i) *The first reduction division in the early oocysts*

To demonstrate the post-zygotic meiosis in the early oocysts of *P. gallinaceum* it was necessary to examine the mid-guts of a large number of mosquitoes at regular intervals during their early infection. Thus, nearly 500 serial slides were prepared from several batches of infected mosquitoes (Tables 1–3). Eventually a complete series showing the different meiotic phases was discovered within a range of 53–55 hr. old oocysts.

Table 1. *The exact time and date of fixation of infected mosquitoes, to discover the first nuclear division of the early oocysts*

| Date | Time of fixation and dissection of mosquitoes | Age of infection | Result |
|---|---|---|---|
| 8. v. 56 | Fed on infected chick at 5 p.m. | Nil | Nil |
| 9. v. 56 | 9 a.m. | 16 hr. | Ookinetes |
| 9. v. 56 | 12 noon | 19 hr. | Many ookinetes |
| 9. v. 56 | 5 p.m. | 24 hr. | Many ookinetes |
| 10. v. 56 | 5 p.m. | 48 hr. | A few ookinetes and tiny uninucleate oocysts |
| 11. v. 56 | 5 p.m. | 72 hr. | Multinucleate oocysts |
| 12. v. 56 | 5 p.m. | 4 days | Multinucleate oocysts showing mitotic division |

Table 2. *The second trial of dissection and fixation of mosquitoes to determine the time for the commencement of first nuclear division in early oocysts*

| Date | Time of fixation and dissection of mosquitoes | Age of infection (hr.) | Result |
|---|---|---|---|
| 22. v. 56 | Fed at 7 a.m. | Nil | Nil |
| 22. v. 56 | 7 a.m. | 24 | Ookinetes |
| 23. v. 56 | 7 p.m. | 36 | Ookinetes |
| 24. v. 56 | 7 a.m. | 48 | Ookinetes and tiny uninucleate oocysts |
| 24. v. 56 | 3 p.m. | 56 | Binucleate oocysts |
| 24. v. 56 | 9 p.m. | 62 | Oocysts with many nuclei |
| 25. v. 56 | 7 a.m. | 72 | Multinucleate oocysts |

(ii) *Nuclear structure in 48–52 hr. old oocysts*

Oocysts were first seen 48 hr. after feeding and a careful study of uninucleate, 48–52 hr. old oocysts showed a clear picture of the nuclear structure at the resting stage. The characteristic nucleus at this stage was similar to that described in ookinetes, except that the chromatin mass was bigger in uninucleate oocysts than in the nucleus of ookinetes (Figs. 8, 9). In stained preparations the oocysts measured about 8–9$\mu$ in diameter.

(iii) *The sequence of meiotic phases in 53–55 hr. old oocysts*

The different meiotic phases were seen in 53–55 hr. old oocysts, and binucleate oocysts were first detected 55 hr. after the first infective meal. The structureless, feebly staining area, which was found around the chromosomes at different meiotic phases, was regarded as a spindle. A similar phenomenon was encountered by Dobell (1925). The first indication of the commencement of the nuclear division was the disappearance of the nuclear membrane, which was followed by the prophase stage.

Table 3. *Showing the dissection and fixation of mosquitoes after 2- and 4-hr. intervals to discover the first reduction division in early oocysts*

| Date | Time of fixation and dissection of mosquitoes | Age of infection (hr.) | Result |
|---|---|---|---|
| 1. vi. 56 | 7 a.m. | Nil | Nil |
| 3. vi. 56 | 7 a.m. | 48 | — |
| 3. vi. 56 | 9 a.m. | 50 | — |
| 3. vi. 56 | 11 a.m. | 52 | — |
| 3. vi. 56 | 12 noon | 53 | — |
| 3. vi. 56 | 1 p.m. | 54 | — |
| 3. vi. 56 | 2 p.m. | 55 | — |
| 3. vi. 56 | 4 p.m. | 57 | — |
| 3. vi. 56 | 7 p.m. | 60 | — |
| 3. vi. 56 | 9 p.m. | 62 | Tetranucleate oocysts |
| 3. vi. 56 | 11 p.m. | 64 | — |
| 4. vi. 56 | 1 a.m. | 66 | — |

*Prophase.* A distinct, stumpy, 'bow-shaped' spireme was observed at early prophase in 53 hr. old oocysts (Fig. 10). This spireme had become condensed, segmented, and gave rise to four diploid chromosomes at middle prophase, as observed in 55 hr. old oocysts (Fig. 11). The two small intensely stained dot-like chromosomes were quite distinguishable from a 'J'-shaped rod, representing the large chromosomes; the second large chromosome was perhaps hidden beneath the upper one. A similar situation was noted by Wolcott (1955). The arrangement of four diploid chromosomes at late prophase is shown in Fig. 12. The size of 53–55 hr. old oocysts was approximately 8·5–10$\mu$.

*Metaphase.* The arrangement of homologous chromosomes in pairs at the equator was seen in 55 hr. old oocysts at this stage; these chromosomes appeared more condensed and stumpy (Fig. 13). The two large chromosomes lay one upon the other, while the two small chromosomes were so closely associated in pairing that only by the difference in focus could they be split into two, revealing an unstained portion in the middle. A clear picture of this phase was seen in a few other oocysts of the same age as illustrated in Fig. 14. Homologous pairs of large and small chromosomes were apparent. This clear picture was probably due to the more extreme flattening of oocysts.

*Anaphase.* The segregation of two homologous groups of haploid chromosomes was observed at early anaphase in oocysts of the same age. Each group was

composed of one large and a small chromosome (Fig. 15). Some of the oocysts showed late anaphase with the haploid groups at the poles and a clear zone had begun to form around them. The presence of the haploid group of chromosomes at this stage indicates that their disintegration had not yet started in order to reconstruct the daughter nuclei as shown in Fig. 16.

*Telophase.* A binucleate condition showing late telophase was seen in 55 hr. old oocysts with distinct nuclear membranes. The chromatin mass of one nucleus showed an irregular outline, while that of the other was nearly spherical, but both contained a central, minute, unstained portion as illustrated in Fig. 17. The nuclear structure at this stage was similar to that described by Grassi (1900) and Wenyon (1926), except that the chromatin threads arising from the karyosome and proceeding towards the nuclear membrane were never found in these early oocysts. These peculiar strands observed by the above authors may be due to the commencement of the formation of an irregular spireme during the early prophase, which was not distinguished by them.

(iv) *The second and following nuclear divisions in tetra- to octonucleate oocysts*

Microscopical examination of longitudinal sections of infected mid-guts and smears of flattened guts showed two distinct stages in 62–65 hr. old oocysts: (i) tetranucleate oocysts; (ii) octonucleate oocysts. The prominent mitotic phases were observed in these oocysts showing the haploid group of chromosomes.

The tetranucleate, 62 hr. old oocysts showing different mitotic phases are shown in Figs. 18–21. During the first mitotic division two daughter nuclei were formed at each pole, and immediately the second mitotic division occurred, giving rise to an octonucleate oocyst. In Fig. 18, the upper nuclei are at prophase showing one curved rod-like and a small deeply stained dot-like structure. These could be regarded as two haploid chromosomes, while the two nuclei at the lower pole are at the resting stage. It was concluded from these observations that the second mitotic division was uneven, as shown in Fig. 20; the one nucleus on the left side of the upper pole is at late anaphase, showing the haploid group of two chromosomes, while the other nuclei are at the resting stage.

Another tetranucleate condition was found in 65 hr. old oocysts as shown in Fig. 22. The two nuclei at the upper pole are at metaphase. Here the two groups of metaphase chromosomes appear more like nuclei than the typical chromosomes, and are seen splitting by the formation of a constriction in the middle. The lower two nuclei are at the resting stage. The number of nuclei varied from 7 to 8 in the oocysts of the same age; this was due to the uneven mitotic division of the nuclei. Some of the nuclei were found at the resting stage, whereas the others were still dividing. This phenomenon is well illustrated in Figs. 23–25. As stated previously, an achromatic figure was represented by a feebly stained zone around the chromosomes at the prominent mitotic phases observed in 62–65 hr. old oocysts.

The shape of the oocysts differed owing to the slight flattening and crushing of the mid-guts. The same pressure may have had an influence on the size of the nuclei, as those lying close to the surface were more flattened and appeared bigger than those more deeply situated. This difference in the size of the nuclei was

observed only in the preparations of smears of mid-guts; in longitudinal sections the nuclei were almost of the same size. Generally, they were found at the poles and near the periphery of the oocysts.

(v) *Multinucleate oocysts*

This stage was seen in 65–72 hr. old oocysts in smears of mid-guts as well as in longitudinal sections (Figs. 26–30). Most of the nuclei were arranged towards the periphery of the oocyst, the rest of the nuclei were scattered, and the dividing nuclei were more clear in sections than in smears at this stage. The number of the nuclei in the oocysts varied from nine to seventeen in the smears and eight to twelve in the sections of mid-guts. The mitotic phases were also prominent at this stage and vacuolation in the oocysts started from the multinucleate stage.

2. *Plasmodium berghei*

(i) *Experimental methods*

Four series of slides were prepared from four different batches of *Anopheles aztecus* fed on infected hamsters on the second and third day after inoculation. These mosquitoes were dissected at 3-, 4- and 1½-hourly intervals from 40 to 60 hr. after the infective feed. The optimum time for the demonstration of meiotic division in the early oocysts of *Plasmodium berghei* was 47½–48½ hr. after the infective feed. Meiosis was completed in one hour, and thus the meiotic division was comparatively more rapid than in the early oocysts of the other species described in this work.

(ii) *Meiotic phases in* 47½–48½ *hr. old oocysts*

The resting phase of the nucleus was seen in 46–47½ hr. old uninucleate oocysts, with a rhomboid-shaped chromatin mass containing a central clear zone, and the whole structure was surrounded by a distinct nuclear membrane. One entire, deeply stained dot observed near the nuclear membrane may be regarded as a centriole (Figs. 34, 35).

*Prophase.* A beaded spireme was observed at prophase in 47½ hr. old oocysts followed by the disappearance of the nuclear membrane, but the entire centriole still persisted (Figs. 36, 37). The size of the oocyst at this stage was 10·6$\mu$.

Four diploid, deeply stained, round, bead-like chromosomes were first seen in 48 hr. old oocysts (Fig. 38). The centriole had divided at this stage. The morphology of chromosomes remained unchanged throughout the different meiotic phases in the early oocysts of *P. berghei*.

*Metaphase.* The equatorial position of four diploid chromosomes was observed in 48 hr. old oocysts showing a well-developed spindle. The different views of metaphase chromosomes are shown in Figs. 39–41. The homologous chromosomes are not so closely associated in pairing as are those in the other species, and have a narrow unstained portion between each pair.

*Anaphase.* The segregation of the haploid group of two chromosomes and its movement towards the poles of the spindle were seen at anaphase in the oocysts of the same age, as shown in Figs. 42–47. The lengthening of the achromatic figure

was noticed at anaphase, followed by its gradual disappearance at telophase (Figs. 47, 48).

*Telophase.* A middle and late telophase stage was detected in 48½ hr. old oocysts, showing the disintegration of the haploid group of chromosomes, and in a binucleate oocyst, respectively (Figs. 48, 49).

(iii) *The second and successive mitotic nuclear divisions in* 49–72 *hr. old oocysts*

The second nuclear division occurred mitotically in 49–50 hr. old oocysts, the haploid group of two chromosomes being clear at prophase and later stages, as shown in Fig. 50. Owing to the successive mitotic divisions of the nuclei a penta- to octo-nucleate condition was seen in 51–53 hr. old oocysts, with the nuclei at various stages of mitosis, as previously described in the case of *P. gallinaceum* (Figs. 51–54). No significant increase was recorded in the size of the oocysts between 50 and 53 hr.

The multinucleate condition was found in 72 hr. old oocysts, showing the uneven mitotic division, as seen in the other species (Fig. 56). These oocysts measured 13·6 $\mu$ in diameter.

### 3. *Plasmodium cynomolgi*

(i) *Experimental methods*

Preparations were made from mid-guts dissected out at hourly intervals from 44 to 72 hr. after the infective feed. The different phases during the first reduction division in the early oocysts of *P. cynomolgi* were observed much more easily owing to the following two facts:

(1) The size of the early oocysts of *P. cynomolgi* was bigger than those in the other species of *Plasmodium* described in this work.

(2) The nucleus was much larger and more distinct.

(ii) *Reduction division in* 48–50 *hr. old oocysts*

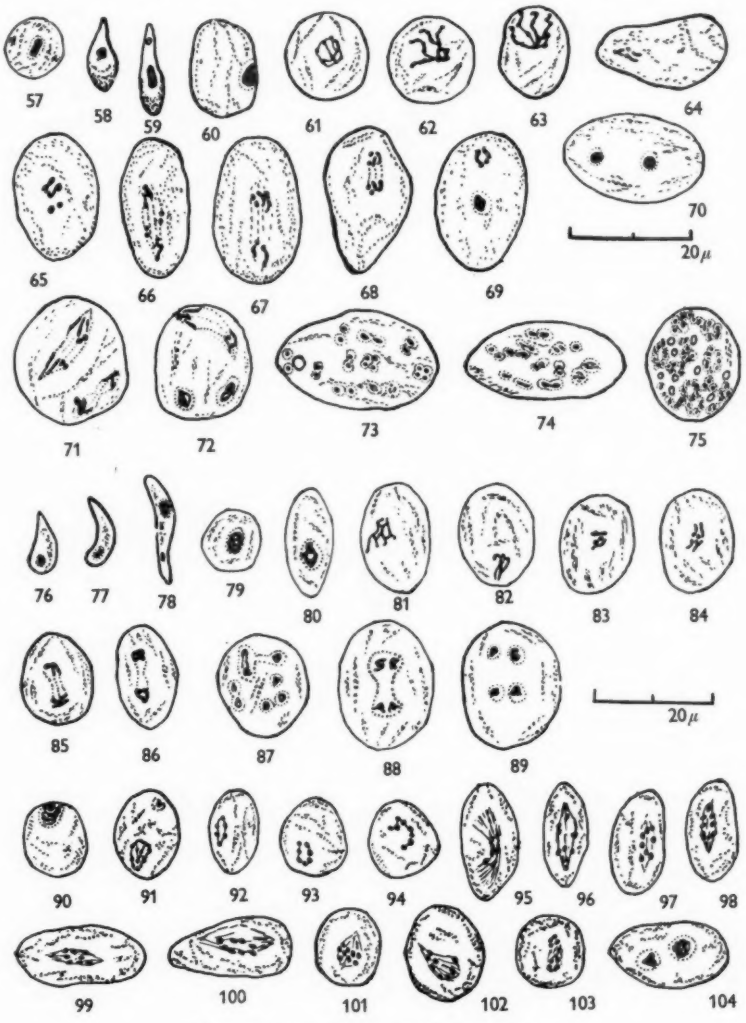
The nucleus was found at the resting stage in 47–48 hr. old uninucleate oocysts as shown in Fig. 60. The sequence of different meitotic phases was observed in 48–50 hr. old oocysts.

*Prophase.* A beaded, filamentous spireme was observed at early prophase in 48 hr. old oocysts and gave rise to eight diploid chromosomes at middle prophase, as seen in 48–50 hr. old oocysts (Figs. 61–63). The two pairs of larger, filamentous chromosomes were seen as if attached to a square composed of the two pairs of small, bead-like chromosomes. At late prophase the four large chromosomes had become more condensed and were easily distinguishable from the four small chromosomes (Fig. 63).

The later meiotic phases were observed in 50 hr. old oocysts and the haploid group was very prominent in the oocysts of the same age. A remarkable change was noticed in the morphology of large chromosomes at the later meiotic phases. They became more condensed and rod-like, while the small chromosomes more or less maintained their shape and size.

*Metaphase.* Two distinct views of metaphase pairing of homologous chromosomes are illustrated in Figs. 64, 65, as seen in 50 hr. old oocysts. In the case of four

large chromosomes, the upper pair appeared to be fused at their upper extremities and one pair of small chromosomes was situated between the two pairs of large chromosomes (Fig. 64). Here, the four small chromosomes give the appearance of a single pair, as in pairing one chromosome probably lay upon another.




Figs. 57–104. For legends see p. 583.

*Anaphase.* The segregation of the haploid group of two large and two small chromosomes was clear at early anaphase (Fig. 66), while each haploid group moved towards the poles of the spindle at middle anaphase, as shown in Fig. 67 of the same plate. The haploid group reached the poles at late anaphase and the chromosomes in this group were easily counted (Figs. 67, 68).

*Telophase.* Finally, telophase was found in 50 hr. old oocysts, giving rise to a binucleate stage (Figs. 69, 70). In Fig. 69, one nucleus is completely formed and at the resting stage, while in the other the haploid group of four chromosomes still remains incompletely fused and distinct prior to the reconstruction of the other daughter nucleus. It is clear that the reconstruction of the daughter nucleus did not occur at the same time in these oocysts of *P. cynomolgi*. The size of 48–50 hr. old oocysts showing different meiotic phases was approximately 14–14·6$\mu$ in diameter.

(iii) *Later successive mitotic divisions of the nuclei in 52–72 hr. old oocysts*

The different mitotic phases were seen in 52–54 hr. old oocysts and the first mitotic division occurred earlier than in *P. gallinaceum*, but later than in *P. berghei*, where it was seen in 62–65 hr. old and 49–50 hr. old oocysts, respectively.

The 52 hr. old oocysts illustrated in Fig. 71 show one nucleus at the upper pole at anaphase with the two haploid groups of chromosomes at the poles and the spindle fibres quite distinct in the middle. The other nucleus is at early telophase. The shape of its spindle is like an 'hour-glass', owing to the formation of a constriction in the middle, and the chromosomes in the haploid group are not so prominent as in the other nucleus. Fig. 72 indicates another phase of mitosis in a 52 hr. old oocyst. The process has been completed, giving rise to two daughter nuclei at the lower pole of the oocyst. Both the nuclei were at the resting stage, while the other nucleus at the upper pole of the oocyst close to the cyst wall was at late anaphase.

As usual a multi-nucleate condition was found in 72 hr. old oocysts of *P. cynomolgi*, showing the prominent mitotic phases, as described in previous species of *Plasmodium*. These nuclei, the majority at the resting phase, numbered 10–12 in each oocyst and were slightly larger than those in *P. gallinaceum* and *P. berghei*; a few vacuoles were present.

### 4. *Plasmodium gonderi*

(i) *Meiotic Phases in 50–54 hr. old oocysts*

At 50 hr. the nucleus of the oocysts was at resting stage and a peculiar pattern of the chromatin mass was visible. This was different from that of the typical resting nucleus described in the early oocysts of the other species. The chromatin of the nucleus consisted of a karyosome composed of an intensely stained peripheral portion and a light-coloured central part. This central zone was divided into small portions by a few thick chromatin strands which arose from the inner surface of the deeply stained peripheral zone. This characteristic form of karyosome at this stage may be due to the fact that the diffusion of the chromatin mass started from its centre and gradually proceeded towards its periphery before the formation of a filamentous spireme (as shown in Figs. 79–81).

*Prophase.* A filamentous spireme was observed in 50 hr. old oocysts, but a period of 4 hr. was required for the differentiation of diploid chromosomes from this stage. This time range of 50–54 hr., between the resting stage and the commencement of prophase, occurred when the infected mosquitoes were kept at a

temperature of 28° C., and 60–65 % relative humidity. After this time lag, the other meiotic phases occurred very rapidly, as illustrated in Figs. 81, 82. The group of four large filamentous and the two rounded, small chromosomes first appeared at late prophase in 54 hr. old oocysts. Thus the diploid complement of chromosomes was six. The two small, round, bead-like chromosomes were closely associated with the curved ends of the two large chromosomes (Fig. 82) and there was no indication of a nuclear membrane at this phase.

*Metaphase.* The cytological arrangement of chromosomes at this phase was seen in 54 hr. old oocysts, as illustrated in Fig. 83, which shows two small chromosomes forming a pair in the middle, while the large chromosomes lay on the right and left sides of the small pair. Only two large chromosomes were visible at this stage; probably the other two were hidden beneath the upper pair.

*Anaphase.* At this stage the large and small chromosomes had already segregated into two distinct haploid groups. Each group consisted of two large and one small chromosomes (Fig. 84). During late anaphase the spindle fibres became more distinguishable and each haploid group of chromosomes was found at the poles, as shown in Fig. 85.

*Telophase.* This stage, observed in 54 hr. old oocysts of *P. gonderi*, differed from the telophase seen in the meiotic division of all the other species of *Plasmodium* studied in that the spindle fibres persisted between the two daughter nuclei. One daughter nucleus was completely reconstructed at one pole, but at the other the process was incomplete and the haploid group of chromosomes was still visible (Fig. 86). In this case the telophase was not completed, for the nuclear membrane had not yet formed around each daughter nucleus. The size of 50–54 hr. old oocysts of *P. gonderi* was about 12$\mu$ in diameter.

(ii) *Later nuclear divisions in 58–72 hr. old oocysts*

The tetranucleate condition, showing the two mitotic phases, was observed in a few 58 hr. old oocysts, as illustrated in Figs. 88, 89. The four daughter nuclei are at late prophase, with the haploid group of two large and one small chromosomes surrounded by a feebly stained 'hour-glass' shaped zone representing the two spindles. The other tetranucleate oocyst in Fig. 89 shows all the nuclei at the resting phase. The size of the nuclei was almost equal but the shape of the chromatin mass varied from triangular, or oval, to spherical.

Observations on the multinucleate condition were based on the study of 72 hr. old oocysts as seen in permanent preparations of entire mid-guts and longitudinal sections of mid-guts. The number of the nuclei in these oocysts was less than that found in *P. cynomolgi* at this age, i.e. not more than five to seven nuclei in each oocyst. The mitotic phases were quite prominent, as shown in Fig. 87. The nucleus on the left shows a typical anaphase arrangement of chromosomes. The group at the upper pole is composed of three haploid chromosomes; the centrally constricted, palely stained zone surrounding these chromosomes may represent the spindle. In another group of three nuclei (as shown in the same figure in the upper part of the oocyst), there is one nucleus, on the right, at the resting stage, while those on the left show anaphase. As usual, the mitotic division at this multinucleate stage is uneven.

## 5. *Plasmodium inui*

### (i) *Experimental methods*

During the course of experimental work, three trials were made in order to detect meiosis in the early oocysts of *P. inui*, using *Anopheles aztecus* and *A. maculipennis atroparvus* as vectors. Three rhesus monkeys (Nos. 169, 184 and 188) were used as vertebrate hosts. Among these only the first monkey had a blood-induced infection; the others were infected by the bites of infected mosquitoes.

Very few infected mosquitoes were available from the first batch on 28 January 1957. Preparations were made at 2-hourly intervals from 64 to 72 hr. after the infective feed. Microscopical examination of the mid-guts revealed a scanty infection and a few oocysts showing the resting phase of the nucleus. The low density of oocysts obtained from this batch made it unsuitable for cytological study of early oocysts.

In the second attempt one batch of *A. aztecus* and two batches of *A. maculipennis atroparvus* were fed on infected monkey No. 184 on 27 and 29 May 1957. The fed mosquitoes were dissected at hourly intervals from 46 to 78 hr. after feeding. Negative results were obtained from the batch of *A. m. atroparvus*. A few meiotic phases were found in the preparations made from the batch of infected *A. aztecus*. The gut infection was very scanty in these mosquitoes and the sequence of different meiotic phases still remained incomplete. It was estimated that the approximate time for the occurrence of meiosis would be within a time range of 72–78 hr. after the infective blood meal.

The infected *A. aztecus* which had survived from the second trial were fed on a clean monkey (No. 188) on 12 and 15 June 1957. This monkey was splenectomized on 31 August 1957. Four days later mature gametocytes appeared in the blood. Three batches of *A. aztecus* were fed on the same monkey on 4, 5 and 6 September 1957. Preparations were made of mid-guts at hourly intervals from 60–96 hr. after feeding. Observations made on 60–66 hr. old preparations revealed ookinetes lying below the elastic membrane. This observation indicated that the penetration of ookinetes was very slow in *Plasmodium inui*. Similar preparations of mid-guts at 67–72 hr. showed rounded up ookinetes which gave rise to uninucleate oocysts.

The first batch of infected mosquitoes showed a fair number of oocysts in the mid-guts, whereas the most successful results were obtained from the second batch. Thus the series of meiotic phases in the early oocysts of *P. inui* was completed.

### (ii) *Meiotic phases in the 72–79 hr. old oocysts*

The earliest recognizable stage in many of the 70 hr. old oocysts was a distinct uninucleate condition showing the resting stage. The structure of the nucleus was similar to that described in the other species (Fig. 90). These oocysts were spherical, measuring approximately 10$\mu$ in diameter.

*Prophase.* A study of 72–79 hr. old oocysts showed the prophase stage and an entire centriole appeared near the periphery for the first time in 72 hr. old oocysts (Fig. 91). A beaded string-like spireme was seen in 72–73 hr. old oocysts at middle prophase, which gave rise to eight diploid, deeply stained, round chromosomes at late prophase, as illustrated in Figs. 92–94.

*Metaphase.* The arrangement of chromosomes at this phase was observed in 74–76 hr. old oocysts. Eight diploid chromosomes were seen scattered on the spindle fibres as if they were preparing for the metaphase position (Figs. 95, 96). The real equatorial position of the diploid group of chromosomes was found in 76 hr. old oocysts, as shown in Figs. 97, 98. Prophase chromosomes were transformed to the metaphase condition after a period of 3 hr.

*Anaphase.* The segregation of the haploid group of chromosomes took place in 76–77 hr. old oocysts. Each haploid group consists of four chromosomes of equal size as seen moving towards the poles along the spindle fibres (Fig. 102). The late anaphase is shown in Fig. 103; the haploid group is at the poles of the spindle.

*Telophase.* Binucleate oocysts showing telophase were observed at 78–79 hr. and these stages are shown in Figs. 104 and 105. The size of 74–79 hr. old oocysts varied between 10·5 and 11$\mu$ in diameter.

(iii) *Later successive mitotic divisions of the nuclei in* 83–96 *hr. old oocysts*

The two daughter nuclei of the binucleate oocysts underwent a mitotic division between 81–83 hr. after the mosquitoes had been fed. This process of mitotic division was uneven in some of the oocysts. The first mitotic division of one daughter nucleus is shown in Fig. 106. In this oocyst, one nucleus on the left is at anaphase showing four distinct haploid chromosomes, while the other, on the right, is still at the resting phase. A study of 83 hr. old oocysts showed both the nuclei in the process of mitotic division, as shown in Fig. 108, and a few oocysts of the same age revealed the telophase stage with faintly visible spindle fibres in the middle (Fig. 109). The diameter of 81–83 hr. old oocysts measured 12–12·6$\mu$.

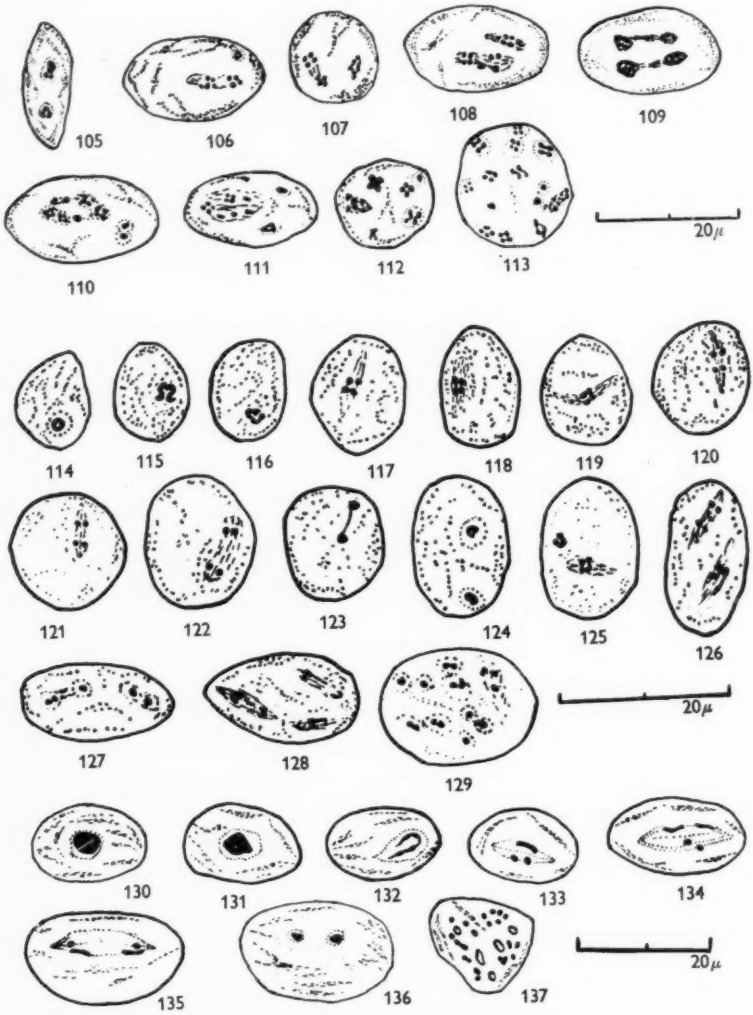
A multinucleate condition was observed in 96–106 hr. old oocysts, showing the prominent mitotic phases as described before in the other species (Figs. 110, 111). In Fig. 110 two nuclei are at late anaphase stage with a distinct haploid group of four chromosomes. In the same figure one nucleus between the two anaphase nuclei, on the right, is at telophase, and another, on the left, is at the resting stage. A similar phenomenon was noticed in other oocysts of the same age, as shown in Fig. 111. The multinucleate condition was more prominent in 106 hr. old oocysts showing an uneven mitotic division of the nuclei (Figs. 112, 113). The morphology of the chromosomes remained unchanged throughout meiotic and mitotic phases. The size of 96–106 hr. old oocysts varied from 13·3 to 15·5$\mu$ in diameter.

### 6. *Plasmodium knowlesi*

(i) *Post-zygotic meiosis in the early oocysts*

During the course of this experimental work a new strain of *P. knowlesi* was used as described by Garnham, Lainson & Cooper (1957). Four attempts were made to infect *Anopheles aztecus* with this strain to carry on the cytological study of the early oocysts. The first attempt gave negative results as mentioned by Garnham *et al.* (1957) and the second trial made on 19 September 1957 gave no better results. In the third attempt, two batches of mosquitoes were fed on a rhesus monkey (M. 192) on the eighth and ninth days after the inoculation on 27 and

28 September 1957, respectively. A scanty gut infection of 50 % of the insects was obtained from the second batch and a few meiotic phases were discovered in mid-gut preparations. It was assumed that the first nuclear division might start in 50 hr. old oocysts. The missing meiotic phases were found in the series prepared




Figs. 105–137. For legends see p. 584.

from the fourth attempt in 50–52 hr. old oocysts. In this trial a monkey (M. 194) was exposed to one batch of *A. aztecus* and a second batch of *A. maculipennis atroparvus* (13 days after the animal was inoculated) on 3 October 1957. Only eight *A. aztecus* were available from the first batch; the density of oocysts was very heavy and the rate of infection was 100 %. Microscopical examination of

stained mid-guts from the second batch of *A. m. atroparvus* revealed a moderate number of oocysts from 50 to 72 hr. after the infective feed. Sixteen mosquitoes were positive out of twenty.

(ii) *Meiotic phases in 51–53 hr. old oocysts*

Uninucleate 49 hr. old oocysts showed a resting phase of the nucleus (Fig. 114). These oocysts were the smallest among the simian species studied, measuring only about 8·5$\mu$ in diameter.

*Prophase.* Oocysts 50 hr. old showed a change in the chromatin mass which took the form of two stumpy 'S'-shaped rods, joined at their distal ends at middle prophase (Fig. 115). This peculiar form of chromatin gave rise to four deeply stained, round, diploid chromosomes at late prophase (Fig. 116).

*Metaphase.* The arrangement of diploid chromosomes at this phase was seen in 51–52 hr. old oocysts showing a well-established spindle (Figs. 117–119). These chromosomes appeared to be dumb-bell shaped at first glance, but careful focusing revealed their proper shape (Figs. 118, 119). The size of the oocysts had increased at this phase and varied from 10·5 to 11·5$\mu$.

*Anaphase.* The segregation of the haploid group of chromosomes was seen in 51 hr. old oocysts at early anaphase, while late anaphase was observed in 52 hr. old oocysts (Figs. 120–122).

*Telophase.* The disintegration of haploid groups of chromosomes (prior to the complete reconstruction of the daughter nuclei) was seen at early telophase in 52 hr. old oocysts, but a few spindle fibres were found joining these two masses of chromatin (Fig. 123). A complete binucleate condition, showing the resting phase of the nuclei, was observed in 53 hr. old oocysts (Fig. 124).

(iii) *Successive mitotic nuclear divisions in 55–72 hr. old oocysts*

The second nuclear division occurred mitotically, as observed in 55–56 hr. old oocysts. The different nuclei undergoing mitosis, as illustrated in Figs. 125–128, do not show the same phase of division. Thus two nuclei in Fig. 125 show an uneven division with the upper nucleus at metaphase, and the lower at prophase. Similarly, Fig. 126 shows the nucleus on the right at anaphase, while the other, to the left, is at metaphase. Another oocyst, illustrated in Fig. 127, gives a picture of nuclei at telophase and at the resting stage. The trinucleate oocyst in Fig. 128 shows all the nuclei undergoing different mitotic phases. As was the case with all the other species, except *Plasmodium inui*, the 72 hr. old oocysts of *P. knowlesi* showed a multinucleate condition. The prominent mitotic phases at this stage were similar to those described in other species (Fig. 129). The diameter of the oocysts at this stage was 14$\mu$.

### 7. *Plasmodium vivax*

A brief description of the different meiotic phases observed in 48 hr. old oocysts of *P. vivax* is as follows.

The resting stage of the nucleus as well as four main meiotic phases were seen in 48 hr. old oocysts. The achromatic figure was visible from metaphase to

anaphase and the number and form of chromosomes was also more prominent at these later meiotic phases. The first nuclear division here occurred earlier than in *P. cynomolgi*.

A striking difference was noticed in the chromatin masses of uninucleate oocysts. In some, the compact mass of chromatin was bordered by a clearly marked nuclear membrane as shown in Fig. 130; in others there was only a very indistinct nuclear membrane about a diffused mass of chromatin (Fig. 131). These uninucleate oocysts gave rise to a beaded 'U'-shaped filamentous spireme at prophase, observed in a few 49 hr. old oocysts (Fig. 132). The equatorial arrangement of homologous chromosomes was seen in the oocysts of the same age, as illustrated in Fig. 133. The two small dot-like chromosomes formed a pair and the two rod-like chromosomes, in pairing, appear as one, presumably owing to superimposition. The segregation of the haploid groups of chromosomes at middle and late anaphase was seen in several oocysts (Fig. 134, 135). These observations showed that the diploid complement of chromosomes is four; two large and two small, and the haploid number is two. These observations confirmed Wolcott's findings (1954–55). Finally, a few binucleate oocysts were seen at telophase, showing the disintegration of the haploid chromosomes and the formation of a nuclear membrane (Fig. 136). The size of 48 hr. old oocysts varied from 11 to 14$\mu$ in diameter.

Owing to the scarcity of infected *Anopheles stephensi* and a high rate of mortality of mosquitoes, further work could not be carried out to determine the earliest age of the oocysts at which a series of tetra- to multinucleate stages could be found. A single surviving mosquito was sectioned to study the cytology of 72 hr. old oocysts. The mitotic division of the nuclei was similar to those described in the previous species of *Plasmodium* such as *P. gallinaceum* and *P. cynomolgi*. These oocysts, however, were more vacuolated, as shown in Fig. 137.

## V. CHARACTERISTIC ARRANGEMENT OF PIGMENT IN THE EARLY OOCYSTS OF SEVEN SPECIES OF *PLASMODIUM*

### 1. *Plasmodium gallinaceum*

The pigment was very coarse and in the form of pepper-like black grains, tending to form a few clumps, or scattered; the number of pigment granules varied from 30 to 40 per oocyst (48–65 hr. old) (Figs. 8–17).

### 2. *Plasmodium berghei*

The fine, blackish brown pigment granules were either arranged in slightly curved streaks crossing each other to form a meshwork, or with a few granules scattered and a few tending to form clumps in 47–72 hr. old oocysts (Figs. 34–56).

### 3. *Plasmodium cynomolgi*

The pigment was of a dark gold colour and took the form of fine rods, forming small streaks in all directions in 48–72 hr. old oocysts (Figs. 60–70).

4. *Plasmodium gonderi*

The pigment was of a dark brown colour and in the form of small, stumpy rods. The streaks formed patterns; thus they were either joined to form a 'U' or 'L' shape as shown in Fig. 82, a 'V' pattern (Figs. 85, 86), or parallel streaks (Figs. 87–89) in the early oocysts.

5. *Plasmodium inui*

The pigment was in the form of fine amber-coloured rods, and showed a tendency to form a streak pattern with some streaks forming a few crosses (Figs. 93–111).

6. *Plasmodium knowlesi*

The pigment in 50–56 hr. old oocysts was in the form of fine dark-brown granules, scattered in the centre of the oocysts but tending also to form streaks near the cyst wall (Figs. 115–127).

7. *Plasmodium vivax*

There was less pigment than in *P. cynomolgi* oocysts; it was light brown in colour and formed very fine rods, which were generally scattered but also tended to form a few streaks (Figs. 132–136).

## VI. DISCUSSION

The zygotes found in the smears of mid-gut contents prepared at half-hourly intervals from 4 to 8 hr. after the infective feed, possessed a single nucleus. Thus the uninucleate condition was detected in the zygotes of the seven species of *Plasmodium* which were studied, i.e. *P. gallinaceum*, *P. berghei*, *P. cynomolgi*, *P. gonderi*, *P. inui*, *P. knowlesi* and *P. vivax*.

The generally described dimorphic ookinetes of human species of *Plasmodium* (Grassi, 1900; Wenyon, 1921; Mackerras & Ercole, 1948) and of *Hepatocystis kochi* (Garnham, 1951*b*) were common in all the seven species of *Plasmodium*. These ookinetes were uninucleate and the nucleus was at the resting stage; the nuclear structure was similar to that described by Grassi (1900), in 2-day-old oocysts of *P. falciparum*. The study of these uninucleate zygotes and ookinetes proved that the first nuclear division did not occur at this stage.

Grassi (1900) was unable to detect the meiotic division as his study was based on 2- to 3-day-old oocysts. According to the present studies, different meiotic phases were detectable between 47 and 60 hr. after the infective feed, except with *P. inui*, in which it occurred after 72 hr. The vital information required for the demonstration of post-zygotic meiosis lies in the determination of the exact time factor.

The sequence of meiotic phases as observed in the early oocysts of the seven species of *Plasmodium* and their specific time factor proved that meiosis is post-zygotic and not pregametic in the genus *Plasmodium*. During the present studies, the different meiotic phases were detected step by step showing the morphology and number of chromosomes and other essential characteristic features of meiosis.

A comparative study of different forms of spireme detected at prophase in seven

species of *Plasmodium* indicated that in each species a special form of spireme is formed which can be regarded as a distinguishing character.

A remarkable change was noticed in the morphology of large chromosomes passing from prophase to metaphase in *P. gallinaceum*, *P. cynomolgi*, *P. gonderi* and *P. vivax*. These large chromosomes were filamentous at prophase, then became more condensed and rod-like from metaphase onwards. A similar phenomenon has been described by Dobell (1925) in *Aggregata eberthi* (coccidian). He observed six vesicular chromosomes at prophase during mitotic division, and these changed to globular forms at metaphase during the same mitotic division. On the other hand, morphologically, the small chromosomes remained unchanged throughout the meiotic and mitotic phases.

The different meiotic phases seen in 53–55 hr. old oocysts of *P. gallinaceum* showed four diploid chromosomes at prophase, while in the later meiotic phases the haploid chromosomes were very prominent. Similarly, in the sequence of mitotic phases seen in binucleate to multinucleate oocysts (55–72 hr. old), prophases were seen with two haploid chromosomes. Study of the early oocysts of six other species of *Plasmodium* showed a phenomenon similar to that described in *P. gallinaceum*; for instance, the prophase stage discovered in 48–50 hr. old oocysts of *P. cynomolgi* showed eight diploid chromosomes, four large and four small. The haploid group of four chromosomes, as seen in the later meiotic phases, remained constant in the prophases of mitosis seen in binucleate to multinucleate, 54–72 hr. old oocysts. The number of chromosomes in the genus *Plasmodium* could not be justified as being constant if discussed on the evolutionary basis, as pointed out by Wolcott (1957).

The evolution of malaria parasites from coccidian-like ancestors has been discussed by Mesnil (1899), Schaudinn (1899), Reichenow (1921), Wenyon (1926) and Manwell (1955), and the number of chromosomes was proved to be a specific character, as described by Dobell (1925). Therefore, the chromosome cycles traced during the present studies were evidently specific characters. Wolcott (1957) showed that a close relationship existed between *P. berghei*, *P. lophurae*, *P. relictum* and *P. floridense* on the basis of their constant number of chromosomes. The results obtained from the cytological study of early oocysts of seven species of *Plasmodium* showed clearly that the genus *Plasmodium* may not always have a complex of two chromosomes. In the present studies a haploid group of two chromosomes was observed during the meiotic and mitotic divisions in the early oocysts of *P. gallinaceum*, *P. berghei*, *P. knowlesi* and *P. vivax*, although the morphology of chromosomes differed. On the other hand, *P. cynomolgi* and *P. gonderi* had a complex of four and three chromosomes, respectively. The haploid number of chromosomes traced in the sequence of meiotic phases and also during the succeeding mitotic divisions in the early oocysts of seven species of *Plasmodium* showed that the number of chromosomes is a specific character and not a generic character.

The second and the following nuclear divisons occurred mitotically in 62–65 hr. old oocysts of *P. gallinaceum* giving rise to tetra- to octo-nucleate oocysts. The haploid group of two chromosomes, one large and one small, was prominent

throughout the different mitotic phases. In *P. cynomolgi* similar succeeding nuclear divisions were seen in 52–54 hr. old oocysts showing the haploid group of four chromosomes, two large and two small.

## VII. SUMMARY AND CONCLUSIONS

1. The cytological study of 4–8 hr. old zygotes of seven species of *Plasmodium*, i.e. *P. gallinaceum*, *P. berghei*, *P. cynomolgi*, *P. gonderi*, *P. inui*, *P. knowlesi* and *P. vivax*, revealed a uninucleate condition at the resting phase.

2. Ookinetes of these species at 12–24 hr were uninucleate and of the usual dimorphic form.

3. In the present studies post-zygotic meiosis was demonstrated in the early oocysts of the above seven species of *Plasmodium*. The times of investigation for the beginning and completion of meiosis in the early oocysts of these species of *Plasmodium* were as follows:

| Parasite | Age of the oocysts showing different meiotic phases (hr.) |
|---|---|
| 1. *P. gallinaceum* | 53–55 |
| 2. *P. berghei* | $47\frac{1}{2}$–$48\frac{1}{2}$ |
| 3. *P. cynomolgi* | 48–50 |
| 4. *P. gonderi* | 50–54 |
| 5. *P. inui* | 72–79 |
| 6. *P. knowlesi* | 51–53 |
| 7. *P. vivax* | 48 |

4. Different forms of spireme at prophases were observed in the early oocysts of the above species of *Plasmodium*. This fact indicated that each species has its own characteristic form of spireme; for instance, 'bow-shaped' in *P. gallinaceum*, beaded, filamentous net-work in *P. cynomolgi*.

5. The diploid and haploid number of chromosomes were demonstrated step by step, during the first reduction division in the early oocysts of seven species of *Plasmodium*.

6. The succeeding nuclear divisions following the post-zygotic meiosis were traced in early oocysts of seven species of *Plasmodium*. The divisions took place mitotically, giving rise to tetra- to multinucleate oocysts. The haploid group of chromosomes in each species was quite distinct during the different mitotic phases found in these oocysts.

7. The results obtained from the cytological study of different meiotic and mitotic phases found in the early oocysts of seven species of *Plasmodium* show that the number of chromosomes is a specific character, for instance, *P. gallinaceum* has a complex of two haploid chromosomes, *P. cynomolgi*, four; *P. gonderi*, three: *P. inui*, four; *P. knowlesi*, *P. berghei* and *P. vivax*, two.

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## KEY TO FIGS. 1–56

All figures were drawn with the aid of camera lucida, under the $\frac{1}{12}$ in. objective; eyepiece $\times 10$; resulting magnification was $\times 1800$. Solid black indicates the chromatin mass or chromosomes; fine dark stippling, the nuclear membrane; coarse black granules, the pigment; irregular stippling for cytoplasm; and fine rods represent the spindle fibres. Figs. 9–17 were drawn from slightly crushed and flattened specimens stained with the modified Wolcott's technique. Figs. 18 and 19 were drawn from longitudinal sections of infected mid-gut. Figs. 22–27 are from entire oocysts in the slightly crushed and flattened stomachs. Figs. 28–30 are from longitudinal sections of infected stomachs. Oocysts in Figs. 18–27, 29 and 30 were stained by Wolcott's technique and oocysts in Figs. 20, 21 and 31 with Heidenhain's iron haematoxylin. Note a peculiar, deeply staining dot in Figs. 34–45, sometimes appearing near the nuclear membrane and considered to represent the centriole; it is entire in Figs. 34–37 and divided in Fig. 38.

### *Plasmodium gallinaceum*

Fig. 1. Division of the chromatin of a microgametocyte of *P. gallinaceum* into six pieces.

Fig. 2. Six flagella emerging from the periphery of a microgametocyte.

Fig. 3. Zygote of *P. gallinaceum*.

Fig. 3A. Young ookinete of *P. gallinaceum*, 16 hr. old, showing one nucleus.

Fig. 3B and D. Ookinetes in the smears of stomach content 19 hr. after feeding. Stained with Giemsa's solution.

Fig. 3C. Retort-shaped ookinete.

Fig. 4. Ookinete, 24 hr. old (curved form), stained by modified Wolcott's technique.

Fig. 5. Ookinete, 24 hr. old, stained with iron haematoxylin (Heidenhain).

Fig. 6. Ookinete, 42 hr. old, stained with iron haematoxylin (Heidenhain). Elongated ookinete.

Fig. 7. Ookinete, 48 hr. old. Pigment present at the anterior end.

Fig. 8. Two-day-old oocyst of *P. gallinaceum*; nucleus at the resting stage. Stained with iron haematoxylin (Heidenhain).

Fig. 9. Uninucleate 52 hr. old oocyst of *P. gallinaceum* showing nucleus at the resting stage.

Fig. 10. 53 hr. old oocyst. Note the disappearance of the nuclear membrane and the appearance of a bow-shaped, stumpy spireme in early prophase.

Fig. 11. 55 hr. old oocyst showing the middle prophase of meiotic division and appearance of chromosomes. The light stippling represents the feebly stained spindle.

Fig. 12. Late prophase seen in a 55 hr. old oocyst.

Figs. 13, 14. Oocyst, 55 hr. old, showing arrangement of chromosomes at metaphase during the first meiotic division.

Fig. 15. Oocyst, of the same age, at early anaphase.

Fig. 16. Arrangement of chromosomes at late anaphase.

Fig. 17. Oocyst, 55 hr. old, presenting telophase of a binucleate oocyst.

Fig. 18. Tetranucleate oocyst, 62 hr. old.

Fig. 19. Another view of tetranucleate oocyst, 62 hr. old.

Fig. 20. Octonucleate oocyst, 62 hr. old; one nucleus at the left side of upper pole dividing and at anaphase.

Fig. 21. Another view of an octonucleate oocyst.

Fig. 22. Entire tetranucleate oocyst, 65 hr. old; two nuclei at the upper pole dividing at metaphase.

Figs. 23–25. Another view of entire, 65 hr. old oocyst showing nuclei at different stages of division.

Fig. 26. 70 hr. old oocyst with sixteen nuclei.

Fig. 27. 70 hr. old oocyst with seven nuclei, at different mitotic phases.

Figs. 28–30. Three-day-old oocyst in longitudinal section of infected mid-gut.

Fig. 31. Three-day-old vacuolated oocyst.

*Plasmodium berghei*

Fig. 32. Late ookinete of *P. berghei*, 47 hr. old.

Fig. 33. Late ookinete of *P. berghei*, 51 hr. old.

Fig. 34. Oocyst, 46½ hr. old, showing a single nucleus at the resting stage.

Fig. 35. Oocyst, 47 hr. old, showing the diffusion of chromatin mass prior to spireme formation.

Fig. 36. Oocyst, 47½ hr. old, with a completely differentiated 'spireme'. Note the absence of the nuclear membrane.

Fig. 37. Oocyst, 47½ hr. old, showing the segmentation of the 'spireme' (prophase).

Fig. 38. Oocyst, 48 hr. old, with four differentiated diploid, round, bead-like chromosomes of equal size.

Fig. 39. Oocyst, of the same age, showing pairing of four homologous chromosomes at the equator of the spindle (early metaphase).

Fig. 40. Oocyst, of the same age, showing another view of the metaphase arrangement of chromosomes.

Fig. 41. Metaphase arrangement of chromosomes showing pairs at the equator of the spindle in 48 hr. old oocyst.

Figs. 42, 43. Two views of the segregation of haploid chromosomes at early anaphase in 48 hr. old oocyst.

Figs. 44, 45. Oocysts showing middle anaphase; note the two haploid groups moving towards the poles of the spindle.

Fig. 46. Oocyst, 48 hr. old, showing haploid grouping of chromosomes at the poles of the spindle (late anaphase).

Fig. 47. Oocysts, of the same age, showing a central constriction in the spindle.

Fig. 48. Oocyst, 48½ hr. old, at telophase.

Fig. 49. Binucleate oocyst at 48½ hr., both nuclei at the resting stage.

Fig. 50. Oocyst, 49 hr. old, showing the first mitotic division in a binucleate oocyst. Note one nucleus at telophase, the other at anaphase.

Fig. 51. Oocysts, 51–52 hr. old, with five nuclei in a process of mitotic division. Note one 'solitary nucleus' close to the periphery of the oocyst.

Fig. 52. Oocyst, of the same age, showing the solitary nucleus at anaphase.

Figs. 53, 54. Oocysts, of the same age, showing the two 'daughter solitary nuclei' near the peripheral region separated from the rest of the nuclei by clumps and streaks of pigment.

Figs. 55, 56. A 3-day-old multinucleate oocyst showing a few mitotically dividing nuclei.

## KEY TO FIGS. 57–104

N.B. Figures 71–75 were drawn from preparations by Wolcott's technique. In Fig. 76–89, the stippling surrounding the nuclei at the resting stage indicates the nuclear membrane, the same stippling in oocysts undergoing nuclear division represents the spindle. The other details are similar to those described for *P. gallinaceum*. Wolcott's staining technique (modified) was used.

### *Plasmodium cynomolgi*

Fig. 57. Zygote of *P. cynomolgi*, 8 hr. old.

Figs. 58, 59. Ookinete 24 hr. after the infective blood meal.

Fig. 60. Uninucleate oocyst, 48 hr. old, showing nucleus at the resting stage.

Fig. 61. Oocyst, 48–50 hr. old, showing nucleus at early prophase. Note the appearance of beaded, filamentous 'spireme'.

Fig. 62. Oocyst of the same age at middle prophase showing eight diploid chromosomes; four large and filamentous, and four small and rounded.

Fig. 63. Oocyst, 48 hr. old; late prophase showing eight distinct diploid chromosomes.

Figs. 64, 65. Oocyst, 50 hr. old, showing pairing of homologous chromosomes at metaphase

Fig. 66. Oocyst, of the same age; segregation of chromosomes at early anaphase.

Fig. 67. Oocyst, of the same age, showing arrangement of two homologous groups of chromosomes at the poles of the spindle at middle anaphase.

Fig. 68. Oocyst, 50 hr. old, showing the chromosome arrangement at late anaphase. Note the haploid group consists of two large and two small chromosomes.

Fig. 69. Oocyst of the same age, at telophase; one daughter nucleus at the lower pole is completely formed. In the other, two large and two small chromosomes are still visible.

Fig. 70. Binucleate oocyst, 50 hr. old, showing both the nuclei at the resting stage.

Fig. 71. Early oocysts of *P. cynomolgi*, 52 hr. old, indicating the second nuclear division (mitotic). Note the upper nucleus is at anaphase and the haploid group of chromosomes at the poles consist of two large and two small chromosomes. The lower nucleus is at telophase. Note a constriction at the centre of the spindle.

Fig. 72. 54 hr. old oocyst showing the upper dividing nucleus at anaphase, the lower two daughter nuclei at the resting stage.

Figs. 73, 74. Longitudinal section of 3-day-old oocyst showing the multinucleate condition.

Fig. 75. Longitudinal section of 4-day-old oocyst. Note the presence of vacuoles and the tendency of the nuclei to form groups.

### *Plasmodium gonderi*

Figs. 76–78. Ookinetes of *P. gonderi*. 21 hr. old.

Figs. 79, 80. Oocyst, 50 hr. old, showing the structure of the nucleus at the resting stage.

Fig. 81. 50 hr. old oocysts. Note the appearance of an irregular filamentous spireme.

Fig. 82. Oocyst, 54 hr. old, showing formation of chromosomes.

Fig. 83. Oocyst, 54 hr. old, with the metaphase arrangement of chromosomes.

Fig. 84. Another view of the oocyst, at the same phase, showing pairs of large and small chromosomes.

Fig. 85. Oocyst, of the same age, showing anaphase arrangement of chromosomes.

Fig. 86. Oocyst, of the same age, showing a peculiar combination of late anaphase and early telophase.

Fig. 87. Three-day-old oocysts at multinucleate stage. Note the nucleus on the left, near the cyst wall, showing anaphase, with a distinct haploid group of chromosomes.

Fig. 88. A binucleate oocyst, 58 hr. old, showing both nuclei at anaphase.

Fig. 89. Tetranucleate oocyst, of the same age, showing all the nuclei at the resting stage.

*Plasmodium inui*

Fig. 90. 70 hr. old, uninucleate oocyst of *P. inui*; note the compact mass of chromatin with clear-cut nuclear membrane.

Fig. 91. Oocyst, 72 hr. old, showing a diffused form of chromatin prior to the formation of a spireme; the appearance of an entire centrosome near the periphery and the disappearance of the nuclear membrane are very striking features.

Figs. 92, 93. Oocyst, of the same age, showing formation of uncoiling beaded, string-like spireme.

Fig. 94. Oocyst, 72 hr. old, showing differentiation of eight diploid chromosomes in the form of eight deeply staining dots.

Figs. 95, 96. Oocysts, 74 hr. old; note the well-established spindle and the densely stained, round bodies representing chromosomes; they are taking up their position on the spindle fibres prior to metaphase arrangement.

Figs. 97, 98. 76 hr. old oocyst, showing metaphase arrangement of eight diploid chromosomes. Note pairing of chromosomes on the spindle fibres.

Figs. 99–101. 76–77 hr. old oocysts giving a picture of the segregation of the haploid group of chromosomes seen moving towards the poles.

Fig. 102. Oocyst, of the same age, showing middle anaphase with the haploid group of four chromosomes passing towards the poles.

Fig. 103. Oocyst from the same age group giving a picture of late anaphase arrangement of chromosomes with the spindle fibres still visible.

Fig. 104. View of 79 hr. old binucleate oocyst showing both nuclei at the resting stage.

## KEY TO FIGS. 105–137

Fig. 105. View of 79 hr. old binucleate oocyst showing both nuclei at the resting stage.

Fig. 106. 81 hr. old oocyst showing the second nuclear division (mitotic). Note that one nucleus, on the right, is at the resting stage, the other on the left, is at anaphase with the haploid group of chromosomes still visible.

Fig. 107. Oocyst, of the same age, showing both nuclei dividing mitotically. The nucleus on the left is at anaphase and the other on the right shows a beaded, string-like spireme.

Fig. 108. 83 hr. old oocyst presenting a similar nuclear division: the nucleus on the right is at the metaphase stage.

Fig. 109. Oocyst, of the same age, showing both nuclei at telophase; note the persisting spindle fibres.

Fig. 110. 96 hr. old oocysts showing uneven mitotic division; note a few nuclei in the process of mitosis, the others at the resting stage.

Fig. 111. Oocyst, of the same age, showing a feebly stained zone surrounding many dividing nuclei. There is a suggestion that the zone may represent the spindles of the dividing nuclei.

*Plasmodium knowlesi*

Figs. 112, 113. Multinucleate, 106 hr. old oocysts showing mitotic division of the nuclei at different phases. At some anaphases four deeply staining dot-like chromosomes are very distinct.

Fig. 114. Uninucleate oocyst, 49 hr. old, showing the nucleus at the resting stage.

Fig. 115. 50 hr. old oocyst showing the change in the chromatin mass which takes the form of two stumpy 'S'-shaped rods, joined at their distal ends.

Fig. 116. Oocyst, of the same age, showing four deeply stained, round diploid chromosomes at late prophase.

Figs. 117–119. 51–52 hr. old oocysts showing metaphase arrangement of four diploid chromosomes of equal size. Note a fully formed spindle.

Figs. 120. Oocyst, of the same age, at early anaphase; note the segregation of chromosomes.

Fig. 121. Oocyst, of the same age, at middle anaphase, the haploid group of two chromosomes is moving towards the poles of the spindle.

Fig. 122. Oocyst, 52 hr. old, showing late anaphase—the haploid group has reached the poles.

Fig. 123. A 52 hr. old oocyst at telophase. A few spindle fibres are still visible and the nuclear membrane is not yet formed.

Fig. 124. A binucleate oocyst showing two daughter nuclei at the resting stage.

Figs. 125–127. Oocysts, 55 and 56 hr. old, showing the second nuclear division (mitotic). In Fig. 125 the upper nucleus is at metaphase and the lower at prophase.

Fig. 126. Oocyst of the same age; the nucleus to the right is at anaphase and the other, to the left, at metaphase.

Fig. 127. Oocyst, 56 hr. old, showing both nuclei at telophase.

Fig. 128. Trinucleate oocyst, 57 hr. old, showing all the nuclei undergoing mitosis.

Fig. 129. Three-day-old oocysts showing a multinucleate condition. Note a few nuclei dividing mitotically.

*Plasmodium vivax*

Fig. 130. Uninucleate oocyst, 48 hr. old, showing a compact mass of chromatin at the resting stage of the nucleus.

Fig. 131. Oocyst, of the same age and stage, showing a diffuse mass of chromatin.

Fig. 132. Oocyst of the same age, showing an 'U'-shaped filamentous spireme.

Fig. 133. Oocyst showing metaphase arrangement of chromosomes. Two are small and dot-like, while two large chromosomes lie one upon the other looking like one. Note the distinct achromatic figure.

Fig. 134. Segregation of the haploid group of chromosomes, one small and one large, in an oocyst of the same age.

Fig. 135. Arrangement of the haploid group of chromosomes at the poles of the spindle.

Fig. 136. A binucleate oocyst, but the haploid group of chromosomes is still visible.

Fig. 137. Longitudinal section of a 3-day-old oocyst in a multinucleate vacuolated stage.

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# A CYTOCHEMICAL INVESTIGATION OF THE SABIN–FELDMAN PHENOMENON IN *TOXOPLASMA GONDII* AND AN EXPLANATION OF ITS MECHANISM ON THIS BASIS

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## INTRODUCTION

The basis of the cytoplasm-modifying test or the dye test (Sabin & Feldman, 1948) is the loss of affinity of live *Toxoplasma* to alkaline methylene blue when incubated at 37° C. for 1 hr. in the presence of an immune serum and normal fresh serum containing a sufficient quantity of the accessory factor. Bringmann & Holz (1953*b*) using the electron-microscope found that the *Toxoplasma* acted on by these antibodies produced a clear picture. In contrast to this they observed in other studies (Bringmann & Holz, 1953*a*; Holz, 1954; Holz & Bringmann, 1954) that normal *Toxoplasma* produced a dense undefined shadow in the electron-microscopic picture. When such *Toxoplasma* were extracted with distilled water at 60° C. they observed that a differentiation in the electron-microscopic picture took place. This was attributed to the extraction of the cytoplasmic ribonucleic acid, the dense shadows being caused by the presence of it. On this basis Bringmann & Holz (1953*a*) explained that the production of a clear electron-microscopic picture after the test was due to the dissolving out of the ribonuclei acid of the cytoplasm. Since this suggested a possible explanation for the Sabin–Feldman phenomenon, a cytological study was undertaken by us to investigate further this observation.

## MATERIALS AND METHODS

The test reaction was carried out in the following manner. The positive serum used was serum from an albino rabbit which had been immunized with repeated injections of macerated mouse brains containing 'pseudocysts' of the avirulent RB 25 strain of *Toxoplasma gondii*. This positive serum, which had a dye-test titre of 1:256 in previous routine tests, was inactivated for $\frac{1}{2}$ hr. in a water-bath at 56° C. just prior to use. The accessory factor serum was nomal human serum which had been found suitable by tests for the absence of dye-test antibodies and for the presence of a sufficient quantity of accessory factor. This serum had been stored for a few weeks in an ice box over solid carbon dioxide (dry ice). It was thawed just before use to minimize the loss of the accessory factor content. The *Toxoplasma* suspension was prepared by adding 0·1 ml. of a 1:500 solution of heparin in saline to each of the peritoneal exudates from 5- to 6-week-old mice. These mice had been infected with 1:10 suspension of peritoneal fluid (each high-power microscopic field containing about fifteen to twenty-five parasites) from a mouse on the third day after infection. Those suspensions which showed a

volume of 0·5–1·0 ml. were first selected, the rest being discarded. Those selected were then microscopically examined for the presence of adequate numbers of *Toxoplasma*. Only exudates which showed over 100 organisms per high-power field were finally selected for use in the test.

For the main test reaction 2 vol. of the positive serum and 4 vol. of the accessory factor serum were added to 1 vol. of the *Toxoplasma* suspension. Four control reactions were also used: (*a*) The negative control, for which the accessory factor serum itself was used as the negative serum. To 6 vol. of the accessory factor serum (2 vol. to represent the negative serum and 4 vol. for the accessory factor serum) was added 1 vol. of the *Toxoplasma* suspension. Since this control contains only the accessory factor serum and the *Toxoplasma* suspension it also acted as an accessory factor control. (*b*) The saline control. To 6 vol. of saline was added 1 vol. of the *Toxoplasma* supsension. (*c*) The positive serum control. For this control 2 vol. of the positive serum, 4 vol. of saline and 1 vol. of the *Toxoplasma* suspension were mixed. (*d*) The incubation control. This control was identical with the saline control as regards volumes and substances. In addition to these controls a normal positive dye test was carried out using the same positive serum diluted serially in fourfold dilutions and containing its own accessory factor control. The same proportions of the accessory factor serum and *Toxoplasma* suspension as in the main positive reaction were added. All the material used in these tests came from the same source, i.e. the *Toxoplasma* suspension was prepared from the peritoneal exudate of one mouse, the accessory factor serum from the same freshly thawed bottle. The contents of the reaction tubes were mixed thoroughly by shaking the tube racks containing them.

*The main test reaction.* Three of the controls, namely, the saline control, the negative serum control and the positive serum control, together with the normal dye test and its accessory factor serum control, were incubated for $\frac{3}{4}$ hr. in a water-bath at 37° C. The incubation control was refrigerated immediately. At the end of the incubation time, the tubes were taken out of the water-bath and 1 vol. of alkaline methylene blue in borate-carbonate buffer at pH 11, which had been prepared a week earlier, was added to each of the test-tubes in the normal dye-test series and to its accessory factor control. The racks containing these tubes were again shaken to mix the dye thoroughly and incubated in the water-bath for a further 5 min. The reaction tubes were then removed from the incubator and refrigerated until the results of the dye test had been evaluated. The cytochemical tests on the positive reaction and the four controls were carried out only if the dye-test results indicated a normal positive reaction.

*The cytochemical studies.* Wet smears of the experimental material were fixed in 80% ethyl alcohol on microscopic slides. The ribonuclease method (Brachet, 1953) was used for detecting the ribonucleic acid, toluidine blue being employed as the basic dye. Protease-free ribonuclease supplied by L. Light and Co. Ltd. was used. For confirmation of the results obtained the method of induced fluorescence as developed by Armstrong (1956) was used with acridine orange in a concentration of 1:2000 in an acetate buffer of pH 4·3–4·8 as the fluorescing agent. The smears were also subjected to the Feulgen reaction. The material was hydrolysed for

5–6 min. at a temperature of 56° C., treated with Schiff's reagent (Coleman, 1938) for 1½ hr., and counterstained with a 0·5 % solution of light green.

## OBSERVATIONS

### (1) *The main reaction*

(*a*) With the toluidine blue stain. The cytoplasm was colourless and the nucleus appeared metachromatic. (*b*) With the fluorescence method. The cytoplasm was devoid of fluorescence but the nucleus showed faint yellow fluorescence. (*c*) The Feulgen reaction. A weak positive reaction was obtained.

### (2) *The negative control (accessory factor control)*

(*a*) With the toluidine blue stain. The nucleus stained blue. Metachromatic granules occurred in the pale blue background of the cytoplasm. (*b*) The fluorescence method. Bright orange fluorescence in the cytoplasm and strong yellowish-green fluorescence in the nucleus were noticed. (*c*) The Feulgen method. The nucleus was faintly Feulgen positive. (*d*) The ribonuclease extraction procedure. The cytoplasm lost its staining property with toluidine blue and the property of fluorescence after being subjected to this method. No such effect was observed in the distilled water controls.

### (3) *The saline control*

All four methods gave the same type of reaction as in the negative control.

### (4) *The positive serum control*

All the results were identical with those of the negative control.

### (5) *The incubation control*

Once again the results were identical with those of the negative control, except that the toluidine blue staining and the fluorescence of the cytoplasm were more intense.

After these studies, modified dye tests were carried out in an attempt to find the influence of blood ribonuclease on the dye test. In the first study a 3 % ribonuclease solution in saline was used instead of the positive serum, the rest of the constituents being the same as in the normal dye test. In the second study the same ribonuclease solution was diluted in saline to make the same volumes as before but without the addition of any serum. The usual controls were employed. In both cases the *Toxoplasma* stained normally with alkaline methylene blue suggesting that the ribonuclease as such has no effect on the live *Toxoplasma* as used in the routine dye test.

## DISCUSSION

Sabin & Feldman (1948) considered three possible explanations for their phenomenon, but found that two were untenable in the presence of contradictory experimental evidence. The first possibility considered was that the failure of the modified *Toxoplasma* to stain with alkaline methylene blue was due to a change which reduced the colour base of the methylene blue to its leuco base. However, when methylene blue was introduced into *Toxoplasma* which were then incubated with antibody and the accessory factor serum, no decolorization of the cytoplasm occurred; further, hydrogen peroxide added to *Toxoplasma* affected by the immunity phenomenon and treated with methylene blue, did not cause the cytoplasm to be stained.

The second possibility considered by Sabin & Feldman (1948) was a change in the permeability of the cell wall of the organism affected by the immunity process. This theory was supported by Westphal & Mühlpfordt (1950) who considered the reaction to be due to a change in the electrical potential relationship of the cell membrane surface caused by a colloidal accumulation of proteins. However, Sabin & Feldman (1948) discarded this point of view as modified *Toxoplasma* exhibited nuclear staining which showed that there was diffusion of the dye into the cytoplasm of the organism. On the other hand, Westphal & Mühlpfordt (1950) believed that only a small amount of dye penetrated into the *Toxoplasma* cell at a time and this deposited on the part of the cell with the greatest affinity, which was the nucleus, leaving the rest of the cell constituents unstained. However, they believed that the staining of the remaining cell constituents would follow in due course. This explanation was supported experimentally. According to them, if a suspension of normal *Toxoplasma* were treated with a quantity of dye inadequate for total dyeing, only the nucleus would be stained and the cytoplasm remain unstained. This dilution was supposed to be reached when two parts of *Toxoplasma* suspension were mixed with four parts of the accessory factor serum and one part of a 1:4 dilution of the methylene blue solution in the accessory serum. As additional support for this interpretation of the mechanism of the dye test, Westphal, Bimmer & Palm (1957) cited the work of Fehrle (see Westphal *et al.* 1957) who established that basic dyes stain normal *Toxoplasma* while acidic dyes behave in exactly the opposite way, a fact already established by Sabin & Feldman (1948). On repeating the experiments we find that, at the concentration suggested by these authors, the staining of the dye-test negative *Toxoplasma* was more prolonged than usual but, within 10–15 min., the staining was sufficiently dense to differentiate the stained from the unstained parasites. The increased time required for the staining should be expected, considering the extreme dilution of the dye in the final solution. Further, according to van Soestbergen (1956) the dye-test readings did not show large variations from the first to the seventh day after the test was carried out. If the theory of semi-permeability is the explanation correct for the mechanism of the dye test, then practically all the *Toxoplasma* should be stained within 15 minutes, which was definitely not the case.

The third possibility put forward by Sabin & Feldman (1948), in the absence

of any other explanation, was a structural change in the groups which have an affinity for the dye. This explanation was not supported by them with any experimental evidence. Lelong & Desmonts (1952) and Desmonts (1954) believed that this process was a modified Pfeiffer's phenomenon on account of the similarity of this process to bacteriolysis (Lelong & Desmonts, 1951, 1952). Grönroos (1955), confirming the observations of Lelong & Desmonts (1951), suggested that the diminished optical density observed was possibly due to loss of substance. Westphal *et al.* (1957) considered that a 90° displacement of phase, which would usually cause a brightening of the picture, could also be caused by an accumulation of an optically dense membrane without *Toxoplasma* undergoing plasmolysis. Pangalos, Pavlatou & Mercier (1955) and Pangalos, Pavlatos & Mercier (1956) using a different technique partly support the observations of Lelong & Desmonts (1951). They consider that the action of the antibodies takes place in two phases. The first phase was the invasion of the cell by the antibodies in combination with the accessory factor which altered the cytoplasm and caused the swelling of the cell. This alteration was responsible for the loss of affinity for the alkaline dye. The second phase was the partial lysis and the expulsion of the cytoplasm in the manner of a plasmolysis.

Bringmann & Holz (1953*b*) found that *Toxoplasma* which were normally dense to the electron rays became clear after the action of the dye-test antibodies. The nucleus was seen clearly contrasted against the clear cytoplasm where electron-scattering particles were present. They assumed on the evidence of an earlier study (Bringmann & Holz, 1953*a*) that this clearing was due to a change in the ribonucleic acid. The electron-scattering particles seen by them were thought to be the metaphosphate accumulations on mitochondria. Braunsteiner, Pakesch & Thalhammer (1957) also reported the presence in *Toxoplasma* of innumerable minute electron-scattering granules which made the structure unclear but disappeared after the dye test. Bringmann & Holz (1953*b*) were not able to establish whether this apparent disappearance of the ribonucleic acid was due to a mere depolymerization or to its complete disappearance. On the grounds of these results they concluded that the action of the immune serum was an interference with the cytoplasmic ribonucleic acid metabolism. The incubation in distilled water at 60° C. is likely to cause the elimination of ribonucleic acid from the cytoplasm as these authors assumed. There does not appear to be sufficient justification for concluding that the substance thus eliminated was ribonucleic acid and ribonucleic acid alone.

On the other hand, Braunsteiner *et al.* (1957) also studied the effect of dye-test antibodies on *Toxoplasma* with the electron microscope using sections. They observed three differences on comparison of dye-test antibody-reacted *Toxoplasma* with those that have not been acted on by the antibody. The first difference seen was that the antibody-acted *Toxoplasma* were not swollen, but small, lancet or sickle shaped in contrast to the unmodified ones. There were no fine dense granules in them as was observed in the normal *Toxoplasma*. The final and most conspicuous observation was the complete lack of their inner structure. The organelles such as the conoid and the toxononemes were replaced by a

homogeneous mass enclosed by the well-preserved outer-cell membrane. The nuclear membrane too was preserved. It was also observed, as by other earlier workers (Bringmann & Holz, 1953*b*; Pangalos *et al.* 1955, 1956), that the change was not complete in all the forms but showed different degrees of change. According to Braunsteiner *et al.* (1957) the antibodies were directed against the proteins of the cell contents and penetrated into the parasite, dissolving them. The penetration of the nuclear membrane took some time. However, the antibodies did penetrate the nuclear barrier and dissolved the nuclear contents. Staining during the time when the nuclear membrane stopped the penetration of the antibodies resulted in the typical picture of the positive Sabin–Feldman test, namely, the unstained transparent *Toxoplasma* with stained nuclei.

From our studies it is clearly seen that a substance which is normally present in *Toxoplasma*, and which has an affinity for toluidine blue and acridine orange in acetate buffer, has disappeared or has lost the affinity for these dyes owing to the action of the antibodies. It is also seen that this same factor could be removed or made to lose its affinity for such dyes by the action of ribonuclease in the fixed condition. If this basophilic factor, that was removed or rendered undemonstrable by ribonuclease, were ribonucleic acid, then it is safe to assume that during the action of the antibodies the ribonucleic acid is changed. On the other hand, it must be pointed out that during the action of 3 % ribonuclease on live *Toxoplasma*, both in the presence of the accessory factor serum and in the absence of it, this basophilic substance was not interfered with. Danielli (1953) put forward certain possibilities for the failure of an enzyme to act on its substrate. For example, completely pure enzyme may not have been utilized, or the enzyme may not obtain access to the substrate, a layer of protein preventing such access. One or both of these reasons may have hindered the action of the ribonuclease in our experiments.

The evidence of Lelong & Desmonts (1951), Bringmann & Holz (1953*b*), Pangalos *et al.* (1955, 1956) and Braunsteiner *et al.* (1957) clearly shows that some structural change has taken place in the *Toxoplasma* owing to the action of the antibodies. Our studies indicate that an identified chemical component, ribonucleic acid (provided the methods by which it was identified are considered specific), has disappeared or been rendered undemonstrable during this immunological process and we consider that this is the cause of the loss of affinity for the alkaline dye. Braunsteiner *et al.* (1957) account for the loss of affinity of modified *Toxoplasma* to the absence of the organelles which normally stain with the dye. However, such minute organelles which could only be seen in the electron microscope could not account for the general staining observed in the dye-test negative *Toxoplasma*. Furthermore, the organelles are expected to be stained more by acidic dyes rather than by strong basic substances like the thiazine dyes. On the other hand, Sabin & Feldman (1948) found that *Toxoplasma* killed by heating at 56° C. for 30 min. exhibited the same lack of affinity for methylene blue, whereas those killed by heating at 50° C. for 15 to 30 min., or by subjecting them to the action of 0·2–0·5 % formalin, retained the affinity for the basic dye. These differences suggest that the loss of affinity is due to the action of an enzyme or to some such biological

substance rather than to a complete breakdown of stainable organelles. Finally, if the two-stage process as was interpreted by Pangalos *et al.* (1955, 1956) is accepted, the loss of stainability occurs in the first phase, which is the disappearance or change in the ribonucleic acid. As the result of further action of the antibodies, or perhaps by autolysis, or by both, a complete breakdown of the cell contents occurs as was seen by Lelong & Desmonts (1951) and Braunsteiner *et al.* (1957). This also explains the observations of the workers using cytological methods who found all degrees of change, whereas in the classical dye test the *Toxoplasma* are either stained or unstained.

## SUMMARY

Ribonucleic acid which was normally present in *Toxoplasma gondii* was found to disappear or become undemonstrable during the action of the dye-test antibodies using cytochemical techniques. A possible explanation for the mechanism of the dye test has been put forward based on this evidence.

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# SOME CYTOCHEMICAL OBSERVATIONS ON *TOXOPLASMA GONDII*

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## INTRODUCTION

Although cytochemical staining reactions in *Toxoplasma gondii* have been recorded by many authors from time to time, these descriptions are widely scattered in the literature. Since no attempt has so far been made to collect these data and to examine them critically, the present study was undertaken for this purpose. Furthermore, some tests were repeated using the old techniques, while others were carried out using newer ones. This was thought to be necessary because none of the previous authors presented a comprehensive account of the reactions obtained by them on the different days of infection, and hence there are gaps in our present knowledge of these reactions which require elucidation.

## HISTORICAL REVIEW

### (*a*) *Deoxyribonucleic acid*

Sassuchin (1933, 1935) reported that *Toxoplasma* found in the smears of the spleen of *Citellus pygmaeus* had Feulgen-positive nuclei. Lillie (1947) found that the vesicular nuclei in the 'Microcysts' ('pseudocysts') of *Toxoplasma* found in sections of the brains of experimentally infected mice were Feulgen-positive. Cross (1947) working on two strains of *Toxoplasma* of human origin maintained in mice, reported a Feulgen-positive reaction in the extracellular and the intracellular forms after very prolonged hydrolysis.

### (*b*) *Ribonucleic acid*

The basophilic material in *Toxoplasma* (origin not mentioned), whicl. was eliminated by hydrochloric acid hydrolysis (Bringmann & Holz, 1953*a*) and also by incubation at 60° C. in double-distilled water, was considered by Bringmann & Holz (1953*a*), Holz (1954) and Holz & Bringmann (1954) to be ribonucleic acid.

### (*c*) *Lipids*

Cross (1947) found finely distributed material which stained with Sudan IV in the clear area of the 'phlange' and in the nuclear matrix of *Toxoplasma*. A negative result was obtained with the osmic-acid method.

### (*d*) *Polysaccharides*

Lillie (1947) noticed in some 'microcysts' ('pseudocysts'), but not in all, many red, slightly purplish, granules in a pale blue matrix when stained according to the Bauer–Feulgen method for glycogen. After digestion with ptyalin and diastase

the reacting material was perhaps more clearly demonstrated, as a deep-purplish red perinuclear deposit, sometimes masking the nucleus, than in the undigested material. Working with lesions found in patients with neonatal toxoplasmosis, Frenkel & Friedlander (1951) reported the occurrence of reacting granules in *Toxoplasma* when stained according to the periodic acid-Schiff method. A few small granules occurred within each organism in the proliferative forms, but in 'pseudocysts' there was a heavy accumulation of such granules, each granule being about the size of the nucleus. The entire cyst stained prominently red, while the cyst wall stained pink. A similar report was later made by Frenkel (1953, 1956). Baar (1957) demonstrated that *Toxoplasma* (of human origin?) stained by the periodic acid-Schiff method, contained reacting material which appeared to be associated with chromatin. This feature was observed in many, but not in all, organisms under observation. Varela, Roch & Vázquez (1955) reported the occurrence of a polysaccharide in a strain of *Toxoplasma* isolated in Mexico (from man or guinea-pig?) using Alcian blue 8GN 150.

## MATERIALS AND METHODS

The parasites of batches A and B were obtained from 5- to 6-weeks'-old white mice each infected with 0·2 ml. of a 1/10 diluted peritoneal exudate (containing about ten to fifteen parasites per high-power field) from a mouse infected 3 days previously with the RH strain of *Toxoplasma gondii*, while those of batches C and D were obtained from 16- to 20-weeks-old mice similarly infected. The smears were prepared from peritoneal exudate of freshly killed mice (batches A and B) or from peritoneal fluid aspirated from mice, with or without saline, depending on the age of the infection (batches C and D). They were fixed immediately (wet fixation) in 80% alcohol. In batch A, all but one mouse was dead by the fifth day of infection, the last one being killed on the sixth day after infection. The majority of the mice in batch B was likewise dead by the fourth day, the last one being killed on the fifth day after the infection. In batches C and D, all mice were dead by the fifth day.

The 'pseudocysts' were obtained from brains of grey mice infected about 4 months previously with the avirulent RB 25 strain of *T. gondii*. The brains were fixed in Carnoy's fluid for ½ hr. immediately after dissecting them out from freshly killed mice. They were embedded in wax and sectioned in the usual way.

The Feulgen reaction for desoxyribonucleic acid was carried out on some of the material which was hydrolyzed using normal hydrochloric acid at 56–58° C. for 5 to 6 min. and then treated with Schiff reagent for 1½ hr.

A part of the material was also stained for 30–45 min. in the prescribed pyronin-methyl green solution for ribonucleic acid. Ribonuclease extraction procedure was followed in all cases with the appropriate distilled water controls. Some of the material was also stained for 10–20 min. in the usual toluidine blue solution. Once again ribonuclease extraction procedure was followed together with the appropriate distilled water controls.

The periodic acid-Schiff reaction was carried out with a little modification. When

the period of exposure to periodic acid solution was extended to 20 min., a better reaction resulted. The treatment with Schiff reagent for 15–45 min. was satisfactory. The Bauer–Feulgen reaction was carried out using 4 % chromic acid and treating with Schiff reagent for 15–45 min. For mucopolysaccharides, Alcian blue 8GS was used. The material was placed in the staining solution for a few seconds only. A longer period of staining resulted in a non-specific reaction in the whole field.

All the above methods were applied to parasites from batches A and B while only the toluidine blue staining method was applied to parasites from batches C and D.

## OBSERVATIONS

### (*a*) *Extracellular forms*

No extracellular forms were seen on the second day of infection in batches A and B. A strongly positive Feulgen reaction was obtained in batch A, but no parasites were seen in batch B on the third day. On the fourth day of infection a weak positive reaction was observed in both batches A and B. However, on the fifth day a strong positive Feulgen reaction was obtained in batch A while a weak positive reaction was recorded in batch B. On the sixth day of infection a weak positive reaction was also observed in batch A.

With the pyronin-methyl green stain certain granules, more deeply stained with pyronin than the cytoplasm of the parasite, appeared to be present in both batches on the third, fourth, fifth and sixth days (batch A only). Methyl green was not usually discernible in the nucleus. Extraction with ribonuclease eliminated this staining property from the cytoplasm.

After staining with toluidine blue no parasites were seen in batch A, while in batch B strong blue staining was observed in the cytoplasm. The nucleus stained faintly blue. In a few parasites the cytoplasm took up a violet shade. In batches A and B on the third and fourth days, the staining observed with toluidine blue was the same as on the previous days. On the fifth day, in batch A the majority had a large number of granules while the others did not. On the sixth day the majority in batch A were without metachromatic granules.

On the second day, in batch C one mouse (*b*) showed the granules, while in batch D none showed such granules. In batch C on the third day, parasites from the same mouse (*b*) showed a large number of metachromatic granules; in parasites from another (*c*) there were no granules while in two others (*a*, *d*) the granules were rare. There were no granules on the third day in parasites from any of the mice of batch D. On the fourth day, in batch C, the parasites from mouse (*a*) showed many metachromatic granules; parasites from mice (*b*) and (*c*) showed few such granules; and mouse (*d*) was dead. In batch D, on the fourth day, only one mouse (*b*) showed large numbers of metachromatic granules; the other mice were dead. Ribonuclease extraction eliminated the metachromatic granules and, broadly speaking, the general basophilia of the cytoplasm.

The parasites on all the days of infection did not appear to stain with Alcian-blue stain.

The reaction with the periodic acid-Schiff method was uncertain with batches A and B on the second day. On the third day of infection positive granules were seen in a few parasites of batch A, while in batch B some had very strongly positive granules and in others such granules were lacking. The results on the fourth day were identical with those of the third day. On the fifth day strongly positive granules were seen in both batches A and B and on the sixth day once again strongly positive granules were seen in batch A.

A very vague pink colour was seen in some specimens of batch A with the Bauer–Feulgen reaction on the fifth day of infection. In all other cases the results were negative.

(*b*) *Intracellular forms*

With batches A and B the parasites on the second day of infection had faintly Feulgen-positive nuclei. In some the reaction was uncertain. On the third day of infection, the nucleus in some was faintly Feulgen-positive, in others it was very strongly positive and in still others the reaction was uncertain. A very poor Feulgen reaction was observed on the fourth day with both batches. On the fifth day of infection a weak reaction was obtained in batch B, while in the other batch a very strong positive reaction was seen. On the sixth day a weak Feulgen reaction was seen in batch A.

In infections of all days, in the smears stained with the pyronin-methyl green technique, the parasites could be very clearly detected in the host cells owing to the intense staining of the *Toxoplasma* cytoplasm. The picture was the same as that shown with the extracellular parasites. Once again, ribonuclease extraction eliminated this staining property.

After staining with toluidine blue the cytoplasm was very deeply stained in most of the parasites on the second day of infection. In some, the cytoplasm exhibited weak metachromasy. The nucleus stained blue but not as strongly as the cytoplasm. On the third and fourth days the reaction was similar to that of the second day of infection. On the fifth day most of the parasites in batch A had metachromatic granules, while in batch B only some of the parasites had these granules. Extraction with ribonuclease eliminated the metachromasy as well as the general basophilia from the cytoplasm.

The parasites of all days of infection did not appear to stain with Alcian-blue stain.

With the periodic acid-Schiff method, on the second day of infection the reaction was uncertain in batch A, while in batch B some had a few positive granules in the cytoplasm. On the third day the parasites in both batches yielded negative results. They were mostly negative on the fourth day and negative in both batches on the fifth day. On the sixth day very strongly positive granules were seen in individuals of batch A.

The Bauer–Feulgen reaction was negative in all cases.

(*c*) '*Pseudocysts*'

The nuclei of the parasites within the 'pseudocysts' were strongly Feulgen-positive. With pyronin-methyl green stain the region of the nucleus showed affinity for both pyronin and methyl green. The rest of the structure was colourless.

After ribonuclease extraction the staining property was largely unaffected except for a slight reduction in the affinity for pyronin in the nuclei.

The result with Alcian blue staining was entirely negative.

With the periodic acid-Schiff reaction, the cytoplasm of the parasites was filled with very strongly reacting granular material. The reaction in the cyst wall exhibited a varying degree of intensity. In some the wall stained only faintly, while in others the entire wall stained intensely. In one cyst only the inner layer of the cyst wall exhibited a very strong positive reaction.

A negative reaction was obtained with the Bauer–Feulgen method.

## DISCUSSION

It is noticeable that in the present study there is some difference in the staining reactions of the parasite at its different stages. For example, the basophilia is negligible or absent in the cytoplasm of the 'pseudocysts' while it is very strongly present in the intracellular and the extracellular forms. However, it is difficult to come to a conclusion on this point. It must be remembered that with regard to the 'pseudocysts' we are dealing with a different strain altogether. The differences noticed in the staining reactions could as well be attributed to strain differences if not to any other cause.

It has also been noticed in the present study that some pronounced differences are seen in the same stages of the parasites obtained at different ages of the infection. The occurrence of metachromatic granules is a case in point. These granules are not seen at all times. It is difficult to ascribe any significance to this finding, although the conclusion that these granules appear only at a late stage of infection is tempting. However, this suggestion finds support in an earlier observation by one of us (Dasgupta, 1958). In this connexion it also appears to be important to take notice of the following. *Toxoplasma* of mouse peritoneal exudate from infections more than 3 days old were found to give a higher percentage of unstained *Toxoplasma* in the dye test than those from 3-day-old infections (Beverley & Beattie, 1952; Cathie & Dudgeon, 1953). Beverley & Beattie (1952) also observed that older mice gave more modified *Toxoplasma* than young ones. According to Grönroos (1955) the same phenomenon was observed in young mice just before they died of *Toxoplasma* infections. Beverley & Beattie (1952) attributed this phenomenon to the presence of antibodies against *Toxoplasma* in the peritoneal fluid. If this is so, then we could expect this appearance of granules to be due to the presence of these antibodies, the different changes noticed being due to differences in the reaction of the individual host to the parasite.

In the present study the ribonuclease method has been employed in trying to demonstrate the presence of ribonucleic acid in *Toxoplasma*. The cytochemical basis of the earlier observation by Bringmann & Holz (1953*a*, *b*), Holz (1954) and Holz & Bringmann (1954), who used hot distilled water and hydrochloric acid for this purpose, does not appear to be very firm. The basophilic substance, including the metachromatic granules, may be considered to represent ribonucleic acid if the specificity of ribonuclease for this purpose is taken for granted.

The variations in the periodic acid-Schiff reaction in the 'pseudocyst' wall, observed in this study, seem to represent different stages in the formation and development of the wall. The failure of Alcian blue to stain any part of this parasite appears to conflict with the earlier report of Varela *et al.* (1955). This contradiction may be attributed to strain differences or may be due to the type of dye used.

It is important to emphasize the fact that this parasite is extremely variable as regards its behaviour to various cytochemical reagents. The cause, or causes, of such variations are far from clear. They may be attributed to strain differences, to differences in the reaction of the host to the parasite, to differences in the period of infection, to different physiological phases of the life-cycle or to some other factor or factors still unknown.

## SUMMARY

A positive Feulgen reaction has been seen in all stages of *Toxoplasma gondii*. The intense basophilia seen in the intracellular and the extracellular stages is removable by treatment with ribonuclease. Granules which reacted positively with the periodic acid-Schiff technique were found in abundance in the cytoplasm of the cysts, whereas the same granules were not universally present in the intracellular and the extracellular stages at all days of infection. The cyst wall stained with this technique with varying degree of intensity.

It is thought that the variations seen in the individual parasites may be attributed to various causes, for example, strain differences, differences in the reaction of the host to the parasite, differences in the period of infection or to other unknown causes.

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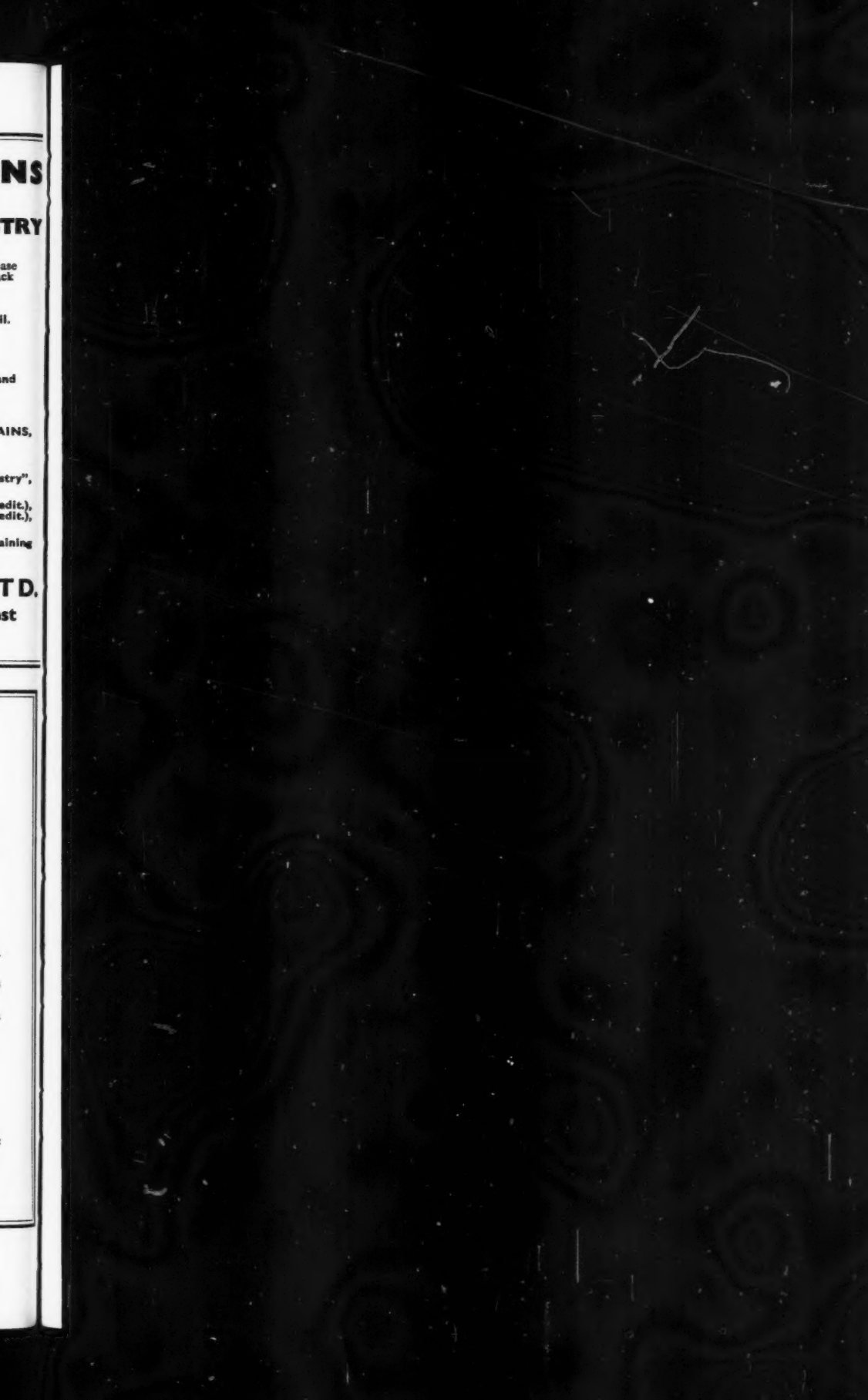

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